



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : <b>C07H 21/04, C07K 1/00, 14/00, C12N 1/21, 15/00, 15/09, 15/63, 15/70, C12P 19/34</b>		<b>A1</b>	(11) International Publication Number: <b>WO 00/52027</b>  (43) International Publication Date: 8 September 2000 (08.09.00)
(21) International Application Number: PCT/US00/05432  (22) International Filing Date: 2 March 2000 (02.03.00)  (30) Priority Data: 60/122,389           2 March 1999 (02.03.99)           US 60/126,049           23 March 1999 (23.03.99)           US 60/136,744           28 May 1999 (28.05.99)           US  (71) Applicant: LIFE TECHNOLOGIES, INC. [US/US]; 9800 Medical Center Drive, Rockville, MD 20850 (US).  (72) Inventors: HARTLEY, James, L.; 7409 Hillside Drive, Frederick, MD 21702 (US). BRASCH, Michael, A.; 20931 Sunnycres Road, Gaithersburg, MD 20882 (US). TEMPLE, Gary, F.; 114 Ridge Road, Washington Grove, MD 20882 (US). CHEO, David; 2006 Baltimore Road, #21, Rockville, MD 20851 (US).  (74) Agents: ESMOND, Robert, W. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>With an indication in relation to deposited biological material furnished under Rule 13bis separately from the description.</i>	
(54) Title: COMPOSITIONS AND METHODS FOR USE IN RECOMBINATIONAL CLONING OF NUCLEIC ACIDS			
(57) Abstract			
<p>The present invention relates generally to compositions and methods for use in recombinational cloning of nucleic acid molecules. In particular, the invention relates to nucleic acid molecules encoding one or more recombination sites or portions thereof, to nucleic acid molecules comprising one or more of these recombination site nucleotide sequences and optionally comprising one or more additional physical or functional nucleotide sequences. The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides using the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof. The invention also relates to the use of these compositions in methods for recombinational cloning of nucleic acids, <i>in vitro</i> and <i>in vivo</i>, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments.</p>			

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## Compositions and Methods for Use in Recombinational Cloning of Nucleic Acids

### BACKGROUND OF THE INVENTION

#### *Field of the Invention*

The present invention relates generally to recombinant DNA technology. More particularly, the present invention relates to compositions and methods for use in recombinational cloning of nucleic acid molecules. The invention relates specifically to nucleic acid molecules encoding one or more recombination sites or one or more partial recombination sites, particularly *attB*, *attP*, *attL*, and *attR*, and fragments, mutants, variants and derivatives thereof. The invention also relates to such nucleic acid molecules wherein the one or more recombination site nucleotide sequences is operably linked to the one or more additional physical or functional nucleotide sequences. The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides and RNAs encoded by the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention, which may be fusion proteins. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof, which may be monoclonal or polyclonal antibodies. The invention also relates to the use of these nucleic acid molecules, vectors, polypeptides and antibodies in methods for recombinational cloning of nucleic acids, *in vitro* and *in vivo*, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments. More particularly, the antibodies of the invention may be used to identify and/or purify proteins or fusion proteins encoded by the nucleic acid molecules or vectors of the invention, or to identify and/or purify the nucleic acid molecules of the invention.

### Related Art

**Site-specific recombinases.** Site-specific recombinases are proteins that are present in many organisms (e.g. viruses and bacteria) and have been characterized to have both endonuclease and ligase properties. These recombinases (along with associated proteins in some cases) recognize specific sequences of bases in DNA and exchange the DNA segments flanking those segments. The recombinases and associated proteins are collectively referred to as "recombination proteins" (see, e.g., Landy, A., *Current Opinion in Biotechnology* 3:699-707 (1993)).

Numerous recombination systems from various organisms have been described. See, e.g., Hoess *et al.*, *Nucleic Acids Research* 14(6):2287 (1986); Abremski *et al.*, *J. Biol. Chem.* 261(1):391 (1986); Campbell, *J. Bacteriol.* 174(23):7495 (1992); Qian *et al.*, *J. Biol. Chem.* 267(11):7794 (1992); Araki *et al.*, *J. Mol. Biol.* 225(1):25 (1992); Maeser and Kahnmann *Mol. Gen. Genet.* 230:170-176 (1991); Esposito *et al.*, *Nucl. Acids Res.* 25(18):3605 (1997).

Many of these belong to the integrase family of recombinases (Argos *et al.*, *EMBO J.* 5:433-440 (1986); Voziyanov *et al.*, *Nucl. Acids Res.* 27:930 (1999)). Perhaps the best studied of these are the Integrase/*att* system from bacteriophage  $\lambda$  (Landy, A. *Current Opinions in Genetics and Devel.* 3:699-707 (1993)), the Cre/*loxP* system from bacteriophage P1 (Hoess and Abremski (1990) In *Nucleic Acids and Molecular Biology*, vol. 4. Eds.: Eckstein and Lilley, Berlin-Heidelberg: Springer-Verlag; pp. 90-109), and the FLP/FRT system from the *Saccharomyces cerevisiae* 2  $\mu$  circle plasmid (Broach *et al.*, *Cell* 29:227-234 (1982)).

Backman (U.S. Patent No. 4,673,640) discloses the *in vivo* use of  $\lambda$  recombinase to recombine a protein producing DNA segment by enzymatic site-specific recombination using wild-type recombination sites *attB* and *attP*.

Hasan and Szybalski (*Gene* 56:145-151 (1987)) discloses the use of  $\lambda$  Int recombinase *in vivo* for intramolecular recombination between wild type *attP* and *attB* sites which flank a promoter. Because the orientations of these sites are



inverted relative to each other, this causes an irreversible flipping of the promoter region relative to the gene of interest.

Palazzolo *et al.* *Gene* 88:25-36 (1990), discloses phage lambda vectors having bacteriophage  $\lambda$  arms that contain restriction sites positioned outside a cloned DNA sequence and between wild-type *loxP* sites. Infection of *E. coli* cells that express the Cre recombinase with these phage vectors results in recombination between the *loxP* sites and the *in vivo* excision of the plasmid replicon, including the cloned cDNA.

Pósfai *et al.* (*Nucl. Acids Res.* 22:2392-2398 (1994)) discloses a method for inserting into genomic DNA partial expression vectors having a selectable marker, flanked by two wild-type FRT recognition sequences. FLP site-specific recombinase as present in the cells is used to integrate the vectors into the genome at predetermined sites. Under conditions where the replicon is functional, this cloned genomic DNA can be amplified.

Bebee *et al.* (U.S. Patent No. 5,434,066) discloses the use of site-specific recombinases such as Cre for DNA containing two *loxP* sites for *in vivo* recombination between the sites.

Boyd (*Nucl. Acids Res.* 21:817-821 (1993)) discloses a method to facilitate the cloning of blunt-ended DNA using conditions that encourage intermolecular ligation to a dephosphorylated vector that contains a wild-type *loxP* site acted upon by a Cre site-specific recombinase present in *E. coli* host cells.

Waterhouse *et al.* (WO 93/19172 and *Nucleic Acids Res.* 21 (9):2265 (1993)) disclose an *in vivo* method where light and heavy chains of a particular antibody were cloned in different phage vectors between *loxP* and *loxP 511* sites and used to transfect new *E. coli* cells. Cre, acting in the host cells on the two parental molecules (one plasmid, one phage), produced four products in equilibrium: two different cointegrates (produced by recombination at either *loxP* or *loxP 511* sites), and two daughter molecules, one of which was the desired product.

Schlake & Bode (*Biochemistry* 33:12746-12751 (1994)) discloses an *in vivo* method to exchange expression cassettes at defined chromosomal locations, each flanked by a wild type and a spacer-mutated FRT recombination site. A

double-reciprocal crossover was mediated in cultured mammalian cells by using this FLP/FRT system for site-specific recombination.

Hartley *et al.* (U.S. Patent No. 5,888,732) disclose compositions and methods for recombinational exchange of nucleic acid segments and molecules, including for use in recombinational cloning of a variety of nucleic acid molecules *in vitro* and *in vivo*, using a combination of wildtype and mutated recombination sites and recombination proteins.

**Transposases.** The family of enzymes, the transposases, has also been used to transfer genetic information between replicons. Transposons are structurally variable, being described as simple or compound, but typically encode the recombinase gene flanked by DNA sequences organized in inverted orientations. Integration of transposons can be random or highly specific. Representatives such as Tn7, which are highly site-specific, have been applied to the *in vivo* movement of DNA segments between replicons (Lucklow *et al.*, *J. Virol.* 67:4566-4579 (1993)).

Devine and Boeke *Nucl. Acids Res.* 22:3765-3772 (1994), discloses the construction of artificial transposons for the insertion of DNA segments, *in vitro*, into recipient DNA molecules. The system makes use of the integrase of yeast TY1 virus-like particles. The DNA segment of interest is cloned, using standard methods, between the ends of the transposon-like element TY1. In the presence of the TY1 integrase, the resulting element integrates randomly into a second target DNA molecule.

**Recombination Sites.** Also key to the integration/recombination reactions mediated by the above-noted recombination proteins and/or transposases are recognition sequences, often termed "recombination sites," on the DNA molecules participating in the integration/recombination reactions. These recombination sites are discrete sections or segments of DNA on the participating nucleic acid molecules that are recognized and bound by the recombination proteins during the initial stages of integration or recombination. For example, the recombination site for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Curr. Opin. Biotech.*

5:521-527 (1994). Other examples of recognition sequences include the *attB*,  
*attP*, *attL*, and *attR* sequences which are recognized by the recombination protein  
 $\lambda$  Int. *attB* is an approximately 25 base pair sequence containing two 9 base pair  
core-type Int binding sites and a 7 base pair overlap region, while *attP* is an  
5 approximately 240 base pair sequence containing core-type Int binding sites and  
arm-type Int binding sites as well as sites for auxiliary proteins integration host  
factor (IHF), FIS and excisionase (Xis). See Landy, *Curr. Opin. Biotech.*  
3:699-707 (1993); see also U.S. Patent No. 5,888,732, which is incorporated by  
reference herein.

10 **DNA cloning.** The cloning of DNA segments currently occurs as a daily  
routine in many research labs and as a prerequisite step in many genetic analyses.  
The purpose of these clonings is various, however, two general purposes can be  
considered: (1) the initial cloning of DNA from large DNA or RNA segments  
(chromosomes, YACs, PCR fragments, mRNA, etc.), done in a relative handful  
15 of known vectors such as pUC, pGem, pBlueScript, and (2) the subcloning of  
these DNA segments into specialized vectors for functional analysis. A great deal  
of time and effort is expended both in the transfer of DNA segments from the  
initial cloning vectors to the more specialized vectors. This transfer is called  
subcloning.

20 The basic methods for cloning have been known for many years and have  
changed little during that time. A typical cloning protocol is as follows:

- (1) digest the DNA of interest with one or two restriction enzymes;
- (2) gel purify the DNA segment of interest when known;
- (3) prepare the vector by cutting with appropriate restriction  
25 enzymes, treating with alkaline phosphatase, gel purify etc., as  
appropriate;
- (4) ligate the DNA segment to the vector, with appropriate  
controls to eliminate background of uncut and self-ligated vector;
- (5) introduce the resulting vector into an *E. coli* host cell;
- 30 (6) pick selected colonies and grow small cultures overnight;
- (7) make DNA minipreps; and

(8) analyze the isolated plasmid on agarose gels (often after diagnostic restriction enzyme digestions) or by PCR.

The specialized vectors used for subcloning DNA segments are functionally diverse. These include but are not limited to: vectors for expressing nucleic acid molecules in various organisms; for regulating nucleic acid molecule expression; for providing tags to aid in protein purification or to allow tracking of proteins in cells; for modifying the cloned DNA segment (*e.g.*, generating deletions); for the synthesis of probes (*e.g.*, riboprobes); for the preparation of templates for DNA sequencing; for the identification of protein coding regions; for the fusion of various protein-coding regions; to provide large amounts of the DNA of interest, *etc.* It is common that a particular investigation will involve subcloning the DNA segment of interest into several different specialized vectors.

As known in the art, simple subclonings can be done in one day (*e.g.*, the DNA segment is not large and the restriction sites are compatible with those of the subcloning vector). However, many other subclonings can take several weeks, especially those involving unknown sequences, long fragments, toxic genes, unsuitable placement of restriction sites, high backgrounds, impure enzymes, *etc.* Subcloning DNA fragments is thus often viewed as a chore to be done as few times as possible.

Several methods for facilitating the cloning of DNA segments have been described, *e.g.*, as in the following references.

Ferguson, J., *et al. Gene* 16:191 (1981), discloses a family of vectors for subcloning fragments of yeast DNA. The vectors encode kanamycin resistance. Clones of longer yeast DNA segments can be partially digested and ligated into the subcloning vectors. If the original cloning vector conveys resistance to ampicillin, no purification is necessary prior to transformation, since the selection will be for kanamycin.

Hashimoto-Gotoh, T., *et al. Gene* 41:125 (1986), discloses a subcloning vector with unique cloning sites within a streptomycin sensitivity gene; in a streptomycin-resistant host, only plasmids with inserts or deletions in the dominant sensitivity gene will survive streptomycin selection.

Accordingly, traditional subcloning methods, using restriction enzymes and ligase, are time consuming and relatively unreliable. Considerable labor is expended, and if two or more days later the desired subclone can not be found among the candidate plasmids, the entire process must then be repeated with alternative conditions attempted. Although site specific recombinases have been used to recombine DNA *in vivo*, the successful use of such enzymes *in vitro* was expected to suffer from several problems. For example, the site specificities and efficiencies were expected to differ *in vitro*; topologically linked products were expected; and the topology of the DNA substrates and recombination proteins was expected to differ significantly *in vitro* (see, e.g., Adams *et al*, *J. Mol. Biol.* 226:661-73 (1992)). Reactions that could go on for many hours *in vivo* were expected to occur in significantly less time *in vitro* before the enzymes became inactive. In addition, the stabilities of the recombination enzymes after incubation for extended periods of time in *in vitro* reactions was unknown, as were the effects of the topologies (*i.e.*, linear, coiled, supercoiled, etc.) of the nucleic acid molecules involved in the reaction. Multiple DNA recombination products were expected in the biological host used, resulting in unsatisfactory reliability, specificity or efficiency of subcloning. Thus, *in vitro* recombination reactions were not expected to be sufficiently efficient to yield the desired levels of product.

Accordingly, there is a long felt need to provide an alternative subcloning system that provides advantages over the known use of restriction enzymes and ligases.

## SUMMARY OF THE INVENTION

The present invention relates to nucleic acid molecules encoding one or more recombination sites or one or more partial recombination sites, particularly *attB*, *attP*, *attL*, and *attR*, and fragments, mutants, variants and derivatives thereof. The invention also relates to such nucleic acid molecules comprising one or more of the recombination site nucleotide sequences or portions thereof and one or more additional physical or functional nucleotide sequences, such as those

encoding one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (*e.g.*, one or more promoters, enhancers, or repressors), one or more translational signal sequences, one or more nucleotide sequences encoding a fusion partner protein or peptide (*e.g.*, GST, His<sub>6</sub>, or thioredoxin), one or more selection markers or modules, one or more nucleotide sequences encoding localization signals such as nuclear localization signals or secretion signals, one or more origins of replication, one or more protease cleavage sites, one or more desired proteins or peptides encoded by a gene or a portion of a gene, and one or more 5' or 3' polynucleotide tails (particularly a poly-G tail). The invention also relates to such nucleic acid molecules wherein the one or more recombination site nucleotide sequences is operably linked to the one or more additional physical or functional nucleotide sequences.

The invention also relates to primer nucleic acid molecules comprising the recombination site nucleotide sequences of the invention (or portions thereof), and to such primer nucleic acid molecules linked to one or more target-specific (*e.g.*, one or more gene-specific) primer nucleic acid sequences. Such primers may also comprise sequences complementary or homologous to DNA or RNA sequences to be amplified, *e.g.*, by PCR, RT-PCR, etc. Such primers may also comprise sequences or portions of sequences useful in the expression of protein genes (ribosome binding sites, localization signals, protease cleavage sites, repressor binding sites, promoters, transcription stops, stop codons, etc.). Said primers may also comprise sequences or portions of sequences useful in the manipulation of DNA molecules (restriction sites, transposition sites, sequencing primers, etc.). The primers of the invention may be used in nucleic acid synthesis and preferably are used for amplification (*e.g.*, PCR) of nucleic acid molecules. When the primers of the invention include target- or gene-specific sequences (any sequence contained within the target to be synthesized or amplified including translation signals, gene sequences, stop codons, transcriptional signals (*e.g.*, promoters) and the like), amplification or synthesis of target sequences or genes may be accomplished. Thus, the invention relates to synthesis of a nucleic acid molecules comprising mixing one or more primers of the invention with a nucleic acid

template, and incubating said mixture under conditions sufficient to make a first nucleic acid molecule complementary to all or a portion of said template. Thus, the invention relates specifically to a method of synthesizing a nucleic acid molecule comprising:

- (a) mixing a nucleic acid template with a polypeptide having polymerase activity and one or more primers comprising one or more recombination sites or portions thereof; and
- (b) incubating said mixture under conditions sufficient to synthesize a first nucleic acid molecule complementary to all or a portion of said template and which preferably comprises one or more recombination sites or portions thereof.

Such method of the invention may further comprise incubating said first synthesized nucleic acid molecule under conditions sufficient to synthesize a second nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule. Such synthesis may provide for a first nucleic acid molecule having a recombination site or portion thereof at one or both of its termini.

In a preferred aspect, for the synthesis of the nucleic acid molecules, at least two primers are used wherein each primer comprises a homologous sequence at its terminus and/or within internal sequences of each primer (which may have a homology length of about 2 to about 500 bases, preferably about 3 to about 100 bases, about 4 to about 50 bases, about 5 to about 25 bases and most preferably about 6 to about 18 base overlap). In a preferred aspect, the first such primer comprises at least one target-specific sequence and at least one recombination site or portion thereof while the second primer comprises at least one recombination site or portion thereof. Preferably, the homologous regions between the first and second primers comprise at least a portion of the recombination site. In another aspect, the homologous regions between the first and second primers may comprise one or more additional sequences, *e.g.*, expression signals, translational start motifs, or other sequences adding functionality to the desired nucleic acid sequence upon amplification. In practice, two pairs of primers prime synthesis or amplification of a nucleic acid molecule. In a preferred aspect, all or at least a portion of the synthesized or amplified nucleic acid molecule will be homologous

to all or a portion of the template and further comprises a recombination site or a portion thereof at at least one terminus and preferably both termini of the synthesized or amplified molecule. Such synthesized or amplified nucleic acid molecule may be double stranded or single stranded and may be used in the recombination cloning methods of the invention. The homologous primers of the invention provide a substantial advantage in that one set of the primers may be standardized for any synthesis or amplification reaction. That is, the primers providing the recombination site sequences (without the target specific sequences) can be pre-made and readily available for use. This in practice allows the use of shorter custom made primers that contain the target specific sequence needed to synthesize or amplify the desired nucleic acid molecule. Thus, this provides reduced time and cost in preparing target specific primers (e.g., shorter primers containing the target specific sequences can be prepared and used in synthesis reactions). The standardized primers, on the other hand, may be produced in mass to reduce cost and can be readily provided (e.g., in kits or as a product) to facilitate synthesis of the desired nucleic acid molecules.

Thus, in one preferred aspect, the invention relates to a method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template specific sequence (complementary to or capable of hybridizing to said templates) and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said second primer is homologous to or complementary to at least a portion of said first primer; and
- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one and preferably both termini of said molecules.



More specifically, the invention relates to a method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template specific sequence (complementary to or capable of hybridizing to said templates) and at least a portion of a recombination site, and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said recombination site on said second primer is complementary to or homologous to at least a portion of said recombination site on said first primer; and
- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one and preferably both termini of said molecules.

In a more preferred aspect, the invention relates to a method of amplifying or synthesizing one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and one or more first primers comprising at least a portion of a recombination site and a template specific sequence (complementary to or capable of hybridizing to said template);
- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more first nucleic acid molecules complementary to all or a portion of said templates wherein said molecules comprise at least a portion of a recombination site at one and preferably both termini of said molecules;
- (c) mixing said molecules with one or more second primers comprising one or more recombination sites, wherein said recombination sites of said second primers are homologous to or

complementary to at least a portion of said recombination sites on said first nucleic acid molecules; and

- (d) incubating said mixture under conditions sufficient to synthesize or amplify one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules and which comprise one or more recombination sites at one and preferably both termini of said molecules.

The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides encoded by the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention, which may be fusion proteins. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof, which may be monoclonal or polyclonal antibodies. The invention also relates to the use of these nucleic acid molecules, primers, vectors, polypeptides and antibodies in methods for recombinational cloning of nucleic acids, *in vitro* and *in vivo*, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments.

The antibodies of the invention may have particular use to identify and/or purify peptides or proteins (including fusion proteins produced by the invention), and to identify and/or purify the nucleic acid molecules of the invention or portions thereof.

The methods for *in vitro* or *in vivo* recombinational cloning of nucleic acid molecule generally relate to recombination between at least a first nucleic acid molecule having at least one recombination site and a second nucleic acid molecule having at least one recombination site to provide a chimeric nucleic acid molecule. In one aspect, the methods relate to recombination between a first vector having at least one recombination site and a second vector having at least one recombination site to provide a chimeric vector. In another aspect, a nucleic acid molecule having at least one recombination site is combined with a vector having at least one recombination site to provide a chimeric vector. In a most preferred aspect, the nucleic acid molecules or vectors used in recombination

comprise two or more recombination sites. In a more specific embodiment of the invention, the recombination methods relate to a Destination Reaction (also referred to herein as an "LR reaction") in which recombination occurs between an Entry clone and a Destination Vector. Such a reaction transfers the nucleic acid molecule of interest from the Entry Clone into the Destination Vector to create an Expression Clone. The methods of the invention also specifically relate to an Entry or Gateward reaction (also referred to herein as a "BP reaction") in which an Expression Clone is recombined with a Donor vector to produce an Entry clone. In other aspects, the invention relates to methods to prepare Entry clones by combining an Entry vector with at least one nucleic acid molecule (e.g., gene or portion of a gene). The invention also relates to conversion of a desired vector into a Destination Vector by including one or more (preferably at least two) recombination sites in the vector of interest. In a more preferred aspect, a nucleic acid molecule (e.g., a cassette) having at least two recombination sites flanking a selectable marker (e.g., a toxic gene or a genetic element preventing the survival of a host cell containing that gene or element, and/or preventing replication, partition or heritability of a nucleic acid molecule (e.g., a vector or plasmid) comprising that gene or element) is added to the vector to make a Destination Vector of the invention.

Preferred vectors for use in the invention include prokaryotic vectors, eukaryotic vectors, or vectors which may shuttle between various prokaryotic and/or eukaryotic systems (e.g. shuttle vectors). Preferred prokaryotic vectors for use in the invention include but are not limited to vectors which may propagate and/or replicate in gram negative and/or gram positive bacteria, including bacteria of the genera *Escherichia*, *Salmonella*, *Proteus*, *Clostridium*, *Klebsiella*, *Bacillus*, *Streptomyces*, and *Pseudomonas* and preferably in the species *E. coli*. Eukaryotic vectors for use in the invention include vectors which propagate and/or replicate and yeast cells, plant cells, mammalian cells, (particularly human and mouse), fungal cells, insect cells, nematode cells, fish cells and the like. Particular vectors of interest include but are not limited to cloning vectors, sequencing vectors, expression vectors, fusion vectors, two-hybrid vectors, gene therapy vectors, phage display vectors, gene-targeting vectors, PACs, BACs, YACs, MACs, and

reverse two-hybrid vectors. Such vectors may be used in prokaryotic and/or eukaryotic systems depending on the particular vector.

In another aspect, the invention relates to kits which may be used in carrying out the methods of the invention, and more specifically relates to cloning or subcloning kits and kits for carrying out the LR Reaction (*e.g.*, making an Expression Clone), for carrying out the BP Reaction (*e.g.*, making an Entry Clone), and for making Entry Clone and Destination Vector molecules of the invention. Such kits may comprise a carrier or receptacle being compartmentalized to receive and hold therein any number of containers. Such containers may contain any number of components for carrying out the methods of the invention or combinations of such components. In particular, a kit of the invention may comprise one or more components (or combinations thereof) selected from the group consisting of one or more recombination proteins or auxiliary factors or combinations thereof, one or more compositions comprising one or more recombination proteins or auxiliary factors or combinations thereof (for example, GATEWAY™ LR Clonase™ Enzyme Mix or GATEWAY™ BP Clonase™ Enzyme Mix), one or more reaction buffers, one or more nucleotides, one or more primers of the invention, one or more restriction enzymes, one or more ligases, one or more polypeptides having polymerase activity (*e.g.*, one or more reverse transcriptases or DNA polymerases), one or more proteinases (*e.g.*, proteinase K or other proteinases), one or more Destination Vector molecules, one or more Entry Clone molecules, one or more host cells (*e.g.* competent cells, such as *E. coli* cells, yeast cells, animal cells (including mammalian cells, insect cells, nematode cells, avian cells, fish cells, etc.), plant cells, and most particularly *E. coli* DB3.1 host cells, such as *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells), instructions for using the kits of the invention (*e.g.*, to carry out the methods of the invention), and the like. In related aspects, the kits of the invention may comprise one or more nucleic acid molecules encoding one or more recombination sites or portions thereof, particularly one or more nucleic acid molecules comprising a nucleotide sequence encoding the one or more recombination sites or portions thereof of the invention. Preferably, such nucleic acid molecules comprise at least two recombination sites which flank a selectable

marker (*e.g.*, a toxic gene and/or antibiotic resistance gene). In a preferred aspect, such nucleic acid molecules are in the form of a cassette (*e.g.*, a linear nucleic acid molecule comprising one or more and preferably two or more recombination sites or portions thereof).

5           Kits for inserting or adding recombination sites to nucleic acid molecules of interest may comprise one or more nucleases (preferably restriction endonucleases), one or more ligases, one or more topoisomerases, one or more polymerases, and one or more nucleic acid molecules or adapters comprising one or more recombination sites. Kits for integrating recombination sites into one or  
10           more nucleic acid molecules of interest may comprise one or more components (or combinations thereof) selected from the group consisting of one or more integration sequences comprising one or more recombination sites. Such integration sequences may comprise one or more transposons, integrating viruses, homologous recombination sequences, RNA molecules, one or more host cells  
15           and the like.

          Kits for making the Entry Clone molecules of the invention may comprise any or a number of components and the composition of such kits may vary depending on the specific method involved. Such methods may involve inserting the nucleic acid molecules of interest into an Entry or Donor Vector by the  
20           recombinational cloning methods of the invention, or using conventional molecular biology techniques (*e.g.*, restriction enzyme digestion and ligation). In a preferred aspect, the Entry Clone is made using nucleic acid amplification or synthesis products. Kits for synthesizing Entry Clone molecules from amplification or synthesis products may comprise one or more components (or combinations  
25           thereof) selected from the group consisting of one or more Donor Vectors (*e.g.*, one or more attP vectors including, but not limited to, pDONR201 (Figure 49), pDONR202 (Figure 50), pDONR203 (Figure 51), pDONR204 (Figure 52), pDONR205 (Figure 53), pDONR206 (Figure 53), and the like), one or more polypeptides having polymerase activity (preferably DNA polymerases and most preferably thermostable DNA polymerases), one or more proteinases, one or more  
30           reaction buffers, one or more nucleotides, one or more primers comprising one or

more recombination sites or portions thereof, and instructions for making one or more Entry Clones.

Kits for making the Destination vectors of the invention may comprise any number of components and the compositions of such kits may vary depending on the specific method involved. Such methods may include the recombination methods of the invention or conventional molecular biology techniques (e.g., restriction endonuclease digestion and ligation). In a preferred aspect, the Destination vector is made by inserting a nucleic acid molecule comprising at least one recombination site (or portion thereof) of the invention (preferably a nucleic acid molecule comprising at least two recombination sites or portions thereof flanking a selectable marker) into a desired vector to convert the desired vector into a Destination vector of the invention. Such kits may comprise at least one component (or combinations thereof) selected from the group consisting of one or more restriction endonucleases, one or more ligases, one or more polymerases, one or more nucleotides, reaction buffers, one or more nucleic acid molecules comprising at least one recombination site or portion thereof (preferably at least one nucleic acid molecule comprising at least two recombination sites flanking at least one selectable marker, such as a cassette comprising at least one selectable marker such as antibiotic resistance genes and/or toxic genes), and instructions for making such Destination vectors.

The invention also relates to kits for using the antibodies of the invention in identification and/or isolation of peptides and proteins (which may be fusion proteins) produced by the nucleic acid molecules of the invention, and for identification and/or isolation of the nucleic acid molecules of the invention or portions thereof. Such kits may comprise one or more components (or combination thereof) selected from the group consisting of one or more antibodies of the invention, one or more detectable labels, one or more solid supports and the like.

Other preferred embodiments of the present invention will be apparent to one of ordinary skill in light of what is known in the art, in light of the following drawings and description of the invention, and in light of the claims.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts one general method of the present invention, wherein the starting (parent) DNA molecules can be circular or linear. The goal is to exchange the new subcloning vector D for the original cloning vector B. It is desirable in one embodiment to select for AD and against all the other molecules, including the Cointegrate. The square and circle are sites of recombination: *e.g.*, *lox* (such as *loxP*) sites, *att* sites, *etc.* For example, segment D can contain expression signals, protein fusion domains, new drug markers, new origins of replication, or specialized functions for mapping or sequencing DNA. It should be noted that the cointegrate molecule contains Segment D (Destination vector) adjacent to segment A (Insert), thereby juxtaposing functional elements in D with the insert in A. Such molecules can be used directly in vitro (*e.g.*, if a promoter is positioned adjacent to a gene-for in vitro transcription/translation) or in vivo (following isolation in a cell capable of propagating *ccdB*-containing vectors) by selecting for the selection markers in Segments B+D. As one skilled in the art will recognize, this single step method has utility in certain envisioned applications of the invention.

Figure 2 is a more detailed depiction of the recombinational cloning system of the invention, referred to herein as the "GATEWAY™ Cloning System." This figure depicts the production of Expression Clones via a "Destination Reaction," which may also be referred to herein as an "LR Reaction." A *kan<sup>r</sup>* vector (referred to herein as an "Entry clone") containing a DNA molecule of interest (*e.g.*, a gene) localized between an *attL1* site and an *attL2* site is reacted with an *amp<sup>r</sup>* vector (referred to herein as a "Destination Vector") containing a toxic or "death" gene localized between an *attR1* site and an *attR2* site, in the presence of GATEWAY™ LR Clonase™ Enzyme Mix (a mixture of Int, IHF and Xis). After incubation at 25°C for about 60 minutes, the reaction yields an *amp<sup>r</sup>* Expression Clone containing the DNA molecule of interest localized between an *attB1* site and an *attB2* site, and a *kan<sup>r</sup>* byproduct molecule, as well as intermediates. The reaction mixture may then be transformed into host cells (*e.g.*, *E. coli*) and clones containing the nucleic acid molecule of interest may

be selected by plating the cells onto ampicillin-containing media and picking amp<sup>r</sup> colonies.

Figure 3 is a schematic depiction of the cloning of a nucleic acid molecule from an Entry clone into multiple types of Destination vectors, to produce a variety of Expression Clones. Recombination between a given Entry clone and different types of Destination vectors (not shown), via the LR Reaction depicted in Figure 2, produces multiple different Expression Clones for use in a variety of applications and host cell types.

Figure 4 is a detailed depiction of the production of Entry Clones via a "BP reaction," also referred to herein as an "Entry Reaction" or a "Gateward Reaction." In the example shown in this figure, an amp<sup>r</sup> expression vector containing a DNA molecule of interest (e.g., a gene) localized between an attB1 site and an attB2 site is reacted with a kan<sup>r</sup> Donor vector (e.g., an attP vector; here, GATEWAY<sup>TM</sup> pDONR201 (see Figure 49A-C)) containing a toxic or "death" gene localized between an attP1 site and an attP2 site, in the presence of GATEWAY<sup>TM</sup> BP Clonase<sup>TM</sup> Enzyme Mix (a mixture of Int and IHF). After incubation at 25°C for about 60 minutes, the reaction yields a kan<sup>r</sup> Entry clone containing the DNA molecule of interest localized between an attL1 site and an attL2 site, and an amp<sup>r</sup> by-product molecule. The Entry clone may then be transformed into host cells (e.g., *E. coli*) and clones containing the Entry clone (and therefore the nucleic acid molecule of interest) may be selected by plating the cells onto kanamycin-containing media and picking kan<sup>r</sup> colonies. Although this figure shows an example of use of a kan<sup>r</sup> Donor vector, it is also possible to use Donor vectors containing other selection markers, such as the gentamycin resistance or tetracycline resistance markers, as discussed herein.

Figure 5 is a more detailed schematic depiction of the LR ("Destination") reaction (Figure 5A) and the BP ("Entry" or "Gateward") reaction (Figure 5B) of the GATEWAY<sup>TM</sup> Cloning System, showing the reactants, products and byproducts of each reaction.



Figure 6 shows the sequences of the attB1 and attB2 sites flanking a gene of interest after subcloning into a Destination Vector to create an Expression Clone.

Figure 7 is a schematic depiction of four ways to make Entry Clones using the compositions and methods of the invention: 1. using restriction enzymes and ligase; 2. starting with a cDNA library prepared in an attL Entry Vector; 3. using an Expression Clone from a library prepared in an attB Expression Vector via the BxP reaction; and 4. recombinational cloning of PCR fragments with terminal attB sites, via the BxP reaction. Approaches 3 and 4 rely on recombination with a Donor vector (here, an attP vector such as pDONR201 (see Figure 49A-C), pDONR202 (see Figure 50A-C), pDONR203 (see Figure 51A-C), pDONR204 (see Figure 52A-C), pDONR205 (see Figure 53A-C), or pDONR206 (see Figure 54A-C), for example) that provides an Entry Clone carrying a selection marker such as kan<sup>r</sup>, gen<sup>r</sup>, tet<sup>r</sup>, or the like.

Figure 8 is a schematic depiction of cloning of a PCR product by a BxP (Entry or Gateward) reaction. A PCR product with 25 bp terminal attB sites (plus four Gs) is shown as a substrate for the BxP reaction. Recombination between the attB-PCR product of a gene and a Donor vector (which donates an Entry Vector that carries kan<sup>r</sup>) results in an Entry Clone of the PCR product.

Figure 9 is a listing of the nucleotide sequences of the recombination sites designated herein as attB1, attB2, attP1, attP2, attL1, attL2, attR1 and attR2. Sequences are written conventionally, from 5' to 3'.

Figures 10-20: The plasmid backbone for all the Entry Vectors depicted herein is the same, and is shown in Figure 10A for the Entry Vector pENTR1A. For other Entry Vectors shown in Figures 11-20, only the sequences shown in Figure "A" for each figure set (i.e., Figure 11A, Figure 12A, etc.) are different (within the attL1-attL2 cassettes) from those shown in Figure 10 -- the plasmid backbone is identical.

Figure 10 is a schematic depiction of the physical map and cloning sites (Figure 10A), and the nucleotide sequence (Figure 10B), of the Entry Vector pENTR1A.

**Figure 11** is a schematic depiction of the cloning sites (Figure 11A) and the nucleotide sequence (Figure 11B) of the Entry Vector pENTR2B.

**Figure 12** is a schematic depiction of the cloning sites (Figure 12A) and the nucleotide sequence (Figure 12B) of the Entry Vector pENTR3C.

5       **Figure 13** is a schematic depiction of the cloning sites (Figure 13A) and the nucleotide sequence (Figure 13B) of the Entry Vector pENTR4.

**Figure 14** is a schematic depiction of the cloning sites (Figure 14A) and the nucleotide sequence (Figure 14B) of the Entry Vector pENTR5.

10       **Figure 15** is a schematic depiction of the cloning sites (Figure 15A) and the nucleotide sequence (Figure 15B) of the Entry Vector pENTR6.

**Figure 16** is a schematic depiction of the cloning sites (Figure 16A) and the nucleotide sequence (Figure 16B) of the Entry Vector pENTR7.

**Figure 17** is a schematic depiction of the cloning sites (Figure 17A) and the nucleotide sequence (Figure 17B) of the Entry Vector pENTR8.

15       **Figure 18** is a schematic depiction of the cloning sites (Figure 18A) and the nucleotide sequence (Figure 18B) of the Entry Vector pENTR9.

**Figure 19** is a schematic depiction of the cloning sites (Figure 19A) and the nucleotide sequence (Figure 19B) of the Entry Vector pENTR10.

20       **Figure 20** is a schematic depiction of the cloning sites (Figure 20A) and the nucleotide sequence (Figure 20B) of the Entry Vector pENTR11.

25       **Figure 21** is a schematic depiction of the physical map and the Trc expression cassette (Figure 21A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 21B-D), of Destination Vector pDEST1. This vector may also be referred to as pTrc-DEST1.

30       **Figure 22** is a schematic depiction of the physical map and the His6 expression cassette (Figure 22A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 22B-D), of Destination Vector pDEST2. This vector may also be referred to as pHis6-DEST2.

**Figure 23** is a schematic depiction of the physical map and the GST expression cassette (Figure 23A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 23B-D), of Destination Vector pDEST3. This vector may also be referred to as pGST-DEST3.

**Figure 24** is a schematic depiction of the physical map and the His6-Trx expression cassette (Figure 24A) showing the promoter sequences at -35 and at -10 from the initiation codon and a TEV protease cleavage site, and the nucleotide sequence (Figure 24B-D), of Destination Vector pDEST4. This vector may also be referred to as pTrx-DEST4.

**Figure 25** is a schematic depiction of the attR1 and attR2 sites (Figure 25A), the physical map (Figure 25B), and the nucleotide sequence (Figure 25C-D), of Destination Vector pDEST5. This vector may also be referred to as pSPORT(+)-DEST5.

**Figure 26** is a schematic depiction of the attR1 and attR2 sites (Figure 26A), the physical map (Figure 26B), and the nucleotide sequence (Figure 26C-D), of Destination Vector pDEST6. This vector may also be referred to as pSPORT(-)-DEST6.

**Figure 27** is a schematic depiction of the attR1 site, CMV promoter, and the physical map (Figure 27A), and the nucleotide sequence (Figure 27B-C), of Destination Vector pDEST7. This vector may also be referred to as pCMV-DEST7.

**Figure 28** is a schematic depiction of the attR1 site, baculovirus polyhedrin promoter, and the physical map (Figure 28A), and the nucleotide sequence (Figure 28B-D), of Destination Vector pDEST8. This vector may also be referred to as pFastBac-DEST8.

**Figure 29** is a schematic depiction of the attR1 site, Semliki Forest Virus promoter, and the physical map (Figure 29A), and the nucleotide sequence (Figure 29B-E), of Destination Vector pDEST9. This vector may also be referred to as pSFV-DEST9.

**Figure 30** is a schematic depiction of the attR1 site, baculovirus polyhedrin promoter, His6 fusion domain, and the physical map (Figure 30A), and the nucleotide sequence (Figure 30B-D), of Destination Vector pDEST10. This vector may also be referred to as pFastBacHT-DEST10.

5 **Figure 31** is a schematic depiction of the attR1 cassette containing a tetracycline-regulated CMV promoter and the physical map (Figure 31A), and the nucleotide sequence (Figure 31B-D), of Destination Vector pDEST11. This vector may also be referred to as pTet-DEST11.

10 **Figure 32** is a schematic depiction of the attR1 site, the start of the mRNA of the CMV promoter, and the physical map (Figure 32A), and the nucleotide sequence (Figure 32B-D), of Destination Vector pDEST12.2. This vector may also be referred to as pCMVneo-DEST12, as pCMV-DEST12, or as pDEST12.

15 **Figure 33** is a schematic depiction of the attR1 site, the  $\lambda P_L$  promoter, and the physical map (Figure 33A), and the nucleotide sequence (Figure 33B-C), of Destination Vector pDEST13. This vector may also be referred to as p $\lambda P_L$ -DEST13.

20 **Figure 34** is a schematic depiction of the attR1 site, the T7 promoter, and the physical map (Figure 34A), and the nucleotide sequence (Figure 34B-D), of Destination Vector pDEST14. This vector may also be referred to as pT7-DEST14.

**Figure 35** is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal GST fusion sequence, and the physical map (Figure 35A), and the nucleotide sequence (Figure 35B-D), of Destination Vector pDEST15. This vector may also be referred to as pT7 GST-DEST15.

25 **Figure 36** is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal thioredoxin fusion sequence, and the physical map (Figure 36A), and the nucleotide sequence (Figure 36B-D), of Destination Vector pDEST16. This vector may also be referred to as pT7 Trx-DEST16.

30 **Figure 37** is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal His6 fusion sequence, and the physical map (Figure 37A), and the

nucleotide sequence (Figure 37B-D), of Destination Vector pDEST17. This vector may also be referred to as pT7 His-DEST17.

5       **Figure 38** is a schematic depiction of the attR1 site and the p10 baculovirus promoter, and the physical map (Figure 38A), and the nucleotide sequence (Figure 38B-D), of Destination Vector pDEST18. This vector may also be referred to as pFBp10-DEST18.

10       **Figure 39** is a schematic depiction of the attR1 site, and the 39k baculovirus promoter, and the physical map (Figure 39A), and the nucleotide sequence (Figure 39B-D), of Destination Vector pDEST19. This vector may also be referred to as pFB39k-DEST19.

**Figure 40** is a schematic depiction of the attR1 site, the *polh* baculovirus promoter, and the N-terminal GST fusion sequence, and the physical map (Figure 40A), and the nucleotide sequence (Figure 40B-D), of Destination Vector pDEST20. This vector may also be referred to as pFB GST-DEST20.

15       **Figure 41** is a schematic depiction of a 2-hybrid vector with a DNA-binding domain, the attR1 site, and the ADH promoter, and the physical map (Figure 41A), and the nucleotide sequence (Figure 41B-E), of Destination Vector pDEST21. This vector may also be referred to as pDB Leu-DEST21.

20       **Figure 42** is a schematic depiction of a 2-hybrid vector with an activation domain, the attR1 site, and the ADH promoter, and the physical map (Figure 42A), and the nucleotide sequence (Figure 42B-D), of Destination Vector pDEST22. This vector may also be referred to as pPC86-DEST22.

25       **Figure 43** is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal His6 fusion sequence, and the physical map (Figure 43A), and the nucleotide sequence (Figure 43B-D), of Destination Vector pDEST23. This vector may also be referred to as pC-term-His6-DEST23.

30       **Figure 44** is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal GST fusion sequence, and the physical map (Figure 44A), and the nucleotide sequence (Figure 44B-D), of Destination Vector pDEST24. This vector may also be referred to as pC-term-GST-DEST24.

Figure 45 is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal thioredoxin fusion sequence, and the physical map (Figure 45A), and the nucleotide sequence (Figure 45B-D), of Destination Vector pDEST25. This vector may also be referred to as pC-term-Trx-DEST25.

5        **Figure 46** is a schematic depiction of the attR1 site, the CMV promoter, and an N-terminal His6 fusion sequence, and the physical map (Figure 46A), and the nucleotide sequence (Figure 46B-D), of Destination Vector pDEST26. This vector may also be referred to as pCMV-SPneo-His-DEST26.

10       **Figure 47** is a schematic depiction of the attR1 site, the CMV promoter, and an N-terminal GST fusion sequence, and the physical map (Figure 47A), and the nucleotide sequence (Figure 47B-D), of Destination Vector pDEST27. This vector may also be referred to as pCMV-Spneo-GST-DEST27.

15       **Figure 48** is a depiction of the physical map (Figure 48A), the cloning sites (Figure 48B), and the nucleotide sequence (Figure 48C-D), for the attB cloning vector plasmid pEXP501. This vector may also be referred to equivalently herein as pCMV•SPORT6, pCMVSPORT6, and pCMVSpport6.

20       **Figure 49** is a depiction of the physical map (Figure 49A), and the nucleotide sequence (Figure 49B-C), for the Donor plasmid pDONR201 which donates a kanamycin-resistant vector in the BP Reaction. This vector may also be referred to as pAttPkanr Donor Plasmid, or as pAttPkan Donor Plasmid

**Figure 50** is a depiction of the physical map (Figure 50A), and the nucleotide sequence (Figure 50B-C), for the Donor plasmid pDONR202 which donates a kanamycin-resistant vector in the BP Reaction.

25       **Figure 51** is a depiction of the physical map (Figure 51A), and the nucleotide sequence (Figure 51B-C), for the Donor plasmid pDONR203 which donates a kanamycin-resistant vector in the BP Reaction.

**Figure 52** is a depiction of the physical map (Figure 52A), and the nucleotide sequence (Figure 52B-C), for the Donor plasmid pDONR204 which donates a kanamycin-resistant vector in the BP Reaction.

Figure 53 is a depiction of the physical map (Figure 53A), and the nucleotide sequence (Figure 53B-C), for the Donor plasmid pDONR205 which donates a tetracycline-resistant vector in the BP Reaction.

5 Figure 54 is a depiction of the physical map (Figure 54A), and the nucleotide sequence (Figure 54B-C), for the Donor plasmid pDONR206 which donates a gentamycin-resistant vector in the BP Reaction. This vector may also be referred to as pENTR22 attP Donor Plasmid, pAttPGenr Donor Plasmid, or pAttPgnt Donor Plasmid.

10 Figure 55 depicts the attB1 site, and the physical map, of an Entry Clone (pENTR7) of CAT subcloned into the Destination Vector pDEST2 (Figure 22).

Figure 56 depicts the DNA components of Reaction B of the one-tube BxP reaction described in Example 16, pEZC7102 and attB-tet-PCR.

Figure 57 is a physical map of the desired product of Reaction B of the one-tube BxP reaction described in Example 16, tetx7102.

15 Figure 58 is a physical map of the Destination Vector pEZC8402.

Figure 59 is a physical map of the expected tet<sup>r</sup> subclone product, tetx8402, resulting from the LxR Reaction with tetx7102 (Figure 57) plus pEZC8402 (Figure 58).

20 Figure 60 is a schematic depiction of the bacteriophage lambda recombination pathways in *E. coli*.

25 Figure 61 is a schematic depiction of the DNA molecules participating in the LR Reaction. Two different co-integrates form during the LR Reaction (only one of which is shown here), depending on whether attL1 and attR1 or attL2 and attR2 are first to recombine. In one aspect, the invention provides directional cloning of a nucleic acid molecule of interest, since the recombination sites react with specificity (attL1 reacts with attR1; attL2 with attR2; attB1 with attP1; and attB2 with attP2). Thus, positioning of the sites allows construction of desired vectors having recombined fragments in the desired orientation.

30 Figure 62 is a depiction of native and fusion protein expression using the recombinational cloning methods and compositions of the invention. In the upper figure depicting native protein expression, all of the translational start signals are

included between the attB1 and attB2 sites; therefore, these signals must be present in the starting Entry Clone. The lower figure depicts fusion protein expression (here showing expression with both N-terminal and C-terminal fusion tags so that ribosomes read through attB1 and attB2 to create the fusion protein). Unlike native protein expression vectors, N-terminal fusion vectors have their

**Figure 63** is a schematic depiction of three GATEWAY™ Cloning System cassettes. Three blunt-ended cassettes are depicted which convert standard expression vectors to Destination Vectors. Each of the depicted cassettes provides amino-terminal fusions in one of three possible reading frames, and each has a distinctive restriction cleavage site as shown.

**Figure 64** shows the physical maps of plasmids containing three attR reading frame cassettes, pEJC15101 (reading frame A; Figure 64A), pEJC15102 (reading frame B; Figure 64B), and pEJC15103 (reading frame C; Figure 64C).

**Figure 65** depicts the attB primers used for amplifying the tet<sup>r</sup> and amp<sup>r</sup> genes from pBR322 by the cloning methods of the invention.

**Figure 66** is a table listing the results of recombinational cloning of the tet<sup>r</sup> and amp<sup>r</sup> PCR products made using the primers shown in Figure 65.

**Figure 67** is a graph showing the effect of the number of guanines (G's) contained on the 5' end of the PCR primers on the cloning efficiency of PCR products. It is noted, however, that other nucleotides besides guanine (including A, T, C, U or combinations thereof) may be used as 5' extensions on the PCR primers to enhance cloning efficiency of PCR products.

**Figure 68** is a graph showing a titration of various amounts of attP and attB reactants in the BxP reaction, and the effects on cloning efficiency of PCR products.

**Figure 69** is a series of graphs showing the effects of various weights (Figure 69A) or moles (Figure 69B) of a 256 bp PCR product on formation of colonies, and on efficiency of cloning of the 256 bp PCR product into a Donor Vector (Figure 69C).



**Figure 70** is a series of graphs showing the effects of various weights (Figure 70A) or moles (Figure 70B) of a 1 kb PCR product on formation of colonies, and on efficiency of cloning of the 1 kb PCR product into a Donor Vector (Figure 70C).

5 **Figure 71** is a series of graphs showing the effects of various weights (Figure 71A) or moles (Figure 71B) of a 1.4 kb PCR product on formation of colonies, and on efficiency of cloning of the 1.4 kb PCR product into a Donor Vector (Figure 71C).

10 **Figure 72** is a series of graphs showing the effects of various weights (Figure 72A) or moles (Figure 72B) of a 3.4 kb PCR product on formation of colonies, and on efficiency of cloning of the 3.4 kb PCR product into a Donor Vector (Figure 72C).

15 **Figure 73** is a series of graphs showing the effects of various weights (Figure 73A) or moles (Figure 73B) of a 4.6 kb PCR product on formation of colonies, and on efficiency of cloning of the 4.6 kb PCR product into a Donor Vector (Figure 73C).

**Figure 74** is photograph of an ethidium bromide-stained gel of a titration of a 6.9 kb PCR product in a BxP reaction.

20 **Figure 75** is a graph showing the effects of various amounts of a 10.1 kb PCR product on formation of colonies upon cloning of the 10.1 kb PCR product into a Donor Vector.

**Figure 76** is photograph of an ethidium bromide-stained gel of a titration of a 10.1 kb PCR product in a BxP reaction.

25 **Figure 77** is a table summarizing the results of the PCR product cloning efficiency experiments depicted in Figures 69-74, for PCR fragments ranging in size from 0.256 kb to 6.9 kb.

30 **Figure 78** is a depiction of the sequences at the ends of attR Cassettes. Sequences contributed by the  $Cm^r$ -ccdB cassette are shown, including the outer ends of the flanking attR sites (boxed). The staggered cleavage sites for Int are indicated in the boxed regions. Following recombination with an Entry Clone, only the outer sequences in attR sites contribute to the resulting attB sites in the

Expression Clone. The underlined sequences at both ends dictate the different reading frames (reading frames A, B, or C, with two alternative reading frame C cassettes depicted) for fusion proteins.

5       **Figure 79** is a depiction of several different attR cassettes (in reading frames A, B, or C) which may provide fusion codons at the amino-terminus of the encoded protein.

**Figure 80** illustrates the single-cutting restriction sites in an attR reading frame A cassette of the invention.

10       **Figure 81** illustrates the single-cutting restriction sites in an attR reading frame B cassette of the invention.

**Figure 82** illustrates the single-cutting restriction sites in two alternative attR reading frame C cassettes of the invention (Figures 82A and 82B) depicted in Figure 78.

15       **Figure 83** shows the physical map (Figure 83A), and the nucleotide sequence (Figure 83B-C), for an attR reading frame C parent plasmid prfC Parent III, which contains an attR reading frame C cassette of the invention (alternative A in Figures 78 and 82).

**Figure 84** is a physical map of plasmid pEZC1301.

**Figure 85** is a physical map of plasmid pEZC1313.

20       **Figure 86** is a physical map of plasmid pEZ14032.

**Figure 87** is a physical map of plasmid pMAB58.

**Figure 88** is a physical map of plasmid pMAB62.

**Figure 89** is a depiction of a synthesis reaction using two pairs of homologous primers of the invention.

25       **Figure 90** is a schematic depiction of the physical map (Figure 90A), and the nucleotide sequence (Figure 90B-D), of Destination Vector pDEST28.

**Figure 91** is a schematic depiction of the physical map (Figure 91A), and the nucleotide sequence (Figure 91B-D), of Destination Vector pDEST29.

30       **Figure 92** is a schematic depiction of the physical map (Figure 92A), and the nucleotide sequence (Figure 92B-D), of Destination Vector pDEST30.

**Figure 93** is a schematic depiction of the physical map (Figure 93A), and the nucleotide sequence (Figure 93B-D), of Destination Vector pDEST31.

**Figure 94** is a schematic depiction of the physical map (Figure 94A), and the nucleotide sequence (Figure 94B-E), of Destination Vector pDEST32.

**Figure 95** is a schematic depiction of the physical map (Figure 95A), and the nucleotide sequence (Figure 95B-D), of Destination Vector pDEST33.

**Figure 96** is a schematic depiction of the physical map (Figure 96A), and the nucleotide sequence (Figure 96B-D), of Destination Vector pDEST34.

**Figure 97** is a depiction of the physical map (Figure 97A), and the nucleotide sequence (Figure 97B-C), for the Donor plasmid pDONR207 which donates a gentamycin-resistant vector in the BP Reaction.

**Figure 98** is a schematic depiction of the physical map (Figure 98A), and the nucleotide sequence (Figure 98B-D), of the 2-hybrid vector pMAB85.

**Figure 99** is a schematic depiction of the physical map (Figure 99A), and the nucleotide sequence (Figure 99B-D), of the 2-hybrid vector pMAB86.

## DETAILED DESCRIPTION OF THE INVENTION

### *Definitions*

In the description that follows, a number of terms used in recombinant DNA technology are utilized extensively. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

**Byproduct:** is a daughter molecule (a new clone produced after the second recombination event during the recombinational cloning process) lacking the segment which is desired to be cloned or subcloned.

**Cointegrate:** is at least one recombination intermediate nucleic acid molecule of the present invention that contains both parental (starting) molecules. It will usually be linear. In some embodiments it can be circular. RNA and polypeptides may be expressed from cointegrates using an appropriate host cell strain, for example *E. coli* DB3.1 (particularly *E. coli* LIBRARY EFFICIENCY®).

DB3.1™ Competent Cells), and selecting for both selection markers found on the cointegrate molecule.

**Host:** is any prokaryotic or eukaryotic organism that can be a recipient of the recombinational cloning Product, vector, or nucleic acid molecule of the invention. A "host," as the term is used herein, includes prokaryotic or eukaryotic organisms that can be genetically engineered. For examples of such hosts, see Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982).

**Insert or Inserts:** include the desired nucleic acid segment or a population of nucleic acid segments (segment *A* of Figure 1) which may be manipulated by the methods of the present invention. Thus, the terms Insert(s) are meant to include a particular nucleic acid (preferably DNA) segment or a population of segments. Such Insert(s) can comprise one or more nucleic acid molecules.

**Insert Donor:** is one of the two parental nucleic acid molecules (e.g. RNA or DNA) of the present invention which carries the Insert. The Insert Donor molecule comprises the Insert flanked on both sides with recombination sites. The Insert Donor can be linear or circular. In one embodiment of the invention, the Insert Donor is a circular DNA molecule and further comprises a cloning vector sequence outside of the recombination signals (see Figure 1). When a population of Inserts or population of nucleic acid segments are used to make the Insert Donor, a population of Insert Donors results and may be used in accordance with the invention. Examples of such Insert Donor molecules are GATEWAY™ Entry Vectors, which include but are not limited to those Entry Vectors depicted in Figures 10-20, as well as other vectors comprising a gene of interest flanked by one or more *attL* sites (e.g., *attL1*, *attL2*, etc.), or by one or more *attB* sites (e.g., *attB1*, *attB2*, etc.) for the production of library clones.

**Product:** is one of the desired daughter molecules comprising the *A* and *D* sequences which is produced after the second recombination event during the recombinational cloning process (see Figure 1). The Product contains the nucleic acid which was to be cloned or subcloned. In accordance with the invention, when a population of Insert Donors are used, the resulting population of Product

molecules will contain all or a portion of the population of Inserts of the Insert Donors and preferably will contain a representative population of the original molecules of the Insert Donors.

**Promoter:** is a DNA sequence generally described as the 5'-region of a gene, located proximal to the start codon. The transcription of an adjacent DNA segment is initiated at the promoter region. A repressible promoter's rate of transcription decreases in response to a repressing agent. An inducible promoter's rate of transcription increases in response to an inducing agent. A constitutive promoter's rate of transcription is not specifically regulated, though it can vary under the influence of general metabolic conditions.

**Recognition sequence:** Recognition sequences are particular sequences which a protein, chemical compound, DNA, or RNA molecule (*e.g.*, restriction endonuclease, a modification methylase, or a recombinase) recognizes and binds. In the present invention, a recognition sequence will usually refer to a recombination site. For example, the recognition sequence for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Current Opinion in Biotechnology* 5:521-527 (1994). Other examples of recognition sequences are the *attB*, *attP*, *attL*, and *attR* sequences which are recognized by the recombinase enzyme  $\lambda$  Integrase. *attB* is an approximately 25 base pair sequence containing two 9 base pair core-type Int binding sites and a 7 base pair overlap region. *attP* is an approximately 240 base pair sequence containing core-type Int binding sites and arm-type Int binding sites as well as sites for auxiliary proteins integration host factor (IHF), FIS and excisionase (Xis). See Landy, *Current Opinion in Biotechnology* 3:699-707 (1993). Such sites may also be engineered according to the present invention to enhance production of products in the methods of the invention. When such engineered sites lack the P1 or H1 domains to make the recombination reactions irreversible (*e.g.*, *attR* or *attP*), such sites may be designated *attR'* or *attP'* to show that the domains of these sites have been modified in some way.

**Recombination proteins:** include excisive or integrative proteins, enzymes, co-factors or associated proteins that are involved in recombination reactions involving one or more recombination sites, which may be wild-type proteins (See Landy, *Current Opinion in Biotechnology* 3:699-707 (1993)), or  
5 mutants, derivatives (e.g., fusion proteins containing the recombination protein sequences or fragments thereof), fragments, and variants thereof.

**Recombination site:** is a recognition sequence on a DNA molecule participating in an integration/recombination reaction by the recombinational cloning methods of the invention. Recombination sites are discrete sections or  
10 segments of DNA on the participating nucleic acid molecules that are recognized and bound by a site-specific recombination protein during the initial stages of integration or recombination. For example, the recombination site for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base  
15 pair core sequence. See Figure 1 of Sauer, B., *Curr. Opin. Biotech.* 5:521-527 (1994). Other examples of recognition sequences include the *attB*, *attP*, *attL*, and *attR* sequences described herein, and mutants, fragments, variants and derivatives thereof, which are recognized by the recombination protein  $\lambda$  Int and by the auxiliary proteins integration host factor (IHF), FIS and excisionase (Xis). See  
20 Landy, *Curr. Opin. Biotech.* 3:699-707 (1993).

**Recombinational Cloning:** is a method described herein, whereby segments of nucleic acid molecules or populations of such molecules are exchanged, inserted, replaced, substituted or modified, *in vitro* or *in vivo*. By  
25 "*in vitro*" and "*in vivo*" herein is meant recombinational cloning that is carried out outside of host cells (e.g., in cell-free systems) or inside of host cells (e.g., using recombination proteins expressed by host cells), respectively.

**Repression cassette:** is a nucleic acid segment that contains a repressor or a Selectable marker present in the subcloning vector.

**Selectable marker:** is a DNA segment that allows one to select for or  
30 against a molecule (e.g., a replicon) or a cell that contains it, often under particular conditions. These markers can encode an activity, such as, but not limited to,

production of RNA, peptide, or protein, or can provide a binding site for RNA, peptides, proteins, inorganic and organic compounds or compositions and the like. Examples of Selectable markers include but are not limited to: (1) DNA segments that encode products which provide resistance against otherwise toxic compounds (e.g., antibiotics); (2) DNA segments that encode products which are otherwise lacking in the recipient cell (e.g., tRNA genes, auxotrophic markers); (3) DNA segments that encode products which suppress the activity of a gene product; (4) DNA segments that encode products which can be readily identified (e.g., phenotypic markers such as  $\beta$ -galactosidase, green fluorescent protein (GFP), and cell surface proteins); (5) DNA segments that bind products which are otherwise detrimental to cell survival and/or function; (6) DNA segments that otherwise inhibit the activity of any of the DNA segments described in Nos. 1-5 above (e.g., antisense oligonucleotides); (7) DNA segments that bind products that modify a substrate (e.g. restriction endonucleases); (8) DNA segments that can be used to isolate or identify a desired molecule (e.g. specific protein binding sites); (9) DNA segments that encode a specific nucleotide sequence which can be otherwise non-functional (e.g., for PCR amplification of subpopulations of molecules); (10) DNA segments, which when absent, directly or indirectly confer resistance or sensitivity to particular compounds; (11) DNA segments that encode products which are toxic in recipient cells; (12) DNA segments that inhibit replication, partition or heritability of nucleic acid molecules that contain them; and/or (13) DNA segments that encode conditional replication functions, e.g., replication in certain hosts or host cell strains or under certain environmental conditions (e.g., temperature, nutritional conditions, etc.).

**Selection scheme:** is any method which allows selection, enrichment, or identification of a desired Product or Product(s) from a mixture containing an Entry Clone or Vector, a Destination Vector, a Donor Vector, an Expression Clone or Vector, any intermediates (e.g. a Cointegrate or a replicon), and/or Byproducts. The selection schemes of one preferred embodiment have at least two components that are either linked or unlinked during recombinational cloning. One component is a Selectable marker. The other component controls the expression *in vitro* or *in vivo* of the Selectable marker, or survival of the cell (or

the nucleic acid molecule, *e.g.*, a replicon) harboring the plasmid carrying the Selectable marker. Generally, this controlling element will be a repressor or inducer of the Selectable marker, but other means for controlling expression or activity of the Selectable marker can be used. Whether a repressor or activator is used will depend on whether the marker is for a positive or negative selection, and the exact arrangement of the various DNA segments, as will be readily apparent to those skilled in the art. A preferred requirement is that the selection scheme results in selection of or enrichment for only one or more desired Products. As defined herein, selecting for a DNA molecule includes (a) selecting or enriching for the presence of the desired DNA molecule, and (b) selecting or enriching against the presence of DNA molecules that are not the desired DNA molecule.

In one embodiment, the selection schemes (which can be carried out in reverse) will take one of three forms, which will be discussed in terms of Figure 1. The first, exemplified herein with a Selectable marker and a repressor therefore, selects for molecules having segment *D* and lacking segment *C*. The second selects against molecules having segment *C* and for molecules having segment *D*. Possible embodiments of the second form would have a DNA segment carrying a gene toxic to cells into which the *in vitro* reaction products are to be introduced. A toxic gene can be a DNA that is expressed as a toxic gene product (a toxic protein or RNA), or can be toxic in and of itself. (In the latter case, the toxic gene is understood to carry its classical definition of "heritable trait".)

Examples of such toxic gene products are well known in the art, and include, but are not limited to, restriction endonucleases (*e.g.*, *DpnI*), apoptosis-related genes (*e.g.* ASK1 or members of the *bcl-2/ced-9* family), retroviral genes including those of the human immunodeficiency virus (HIV), defensins such as NP-1, inverted repeats or paired palindromic DNA sequences, bacteriophage lytic genes such as those from  $\Phi$ X174 or bacteriophage T4; antibiotic sensitivity genes such as *rpsL*, antimicrobial sensitivity genes such as *pheS*, plasmid killer genes, eukaryotic transcriptional vector genes that produce a gene product toxic to bacteria, such as GATA-1, and genes that kill hosts in the absence of a suppressing function, *e.g.*, *kicB*, *ccdB*,  $\Phi$ X174 *E* (Liu, Q. *et al.*, *Curr. Biol.*



8:1300-1309 (1998)), and other genes that negatively affect replicon stability and/or replication. A toxic gene can alternatively be selectable *in vitro*, e.g., a restriction site.

Many genes coding for restriction endonucleases operably linked to inducible promoters are known, and may be used in the present invention. See, e.g. U.S. Patent Nos. 4,960,707 (*DpnI* and *DpnII*); 5,000,333, 5,082,784 and 5,192,675 (*KpnI*); 5,147,800 (*NgoAIII* and *NgoAI*); 5,179,015 (*FspI* and *HaeIII*); 5,200,333 (*HaeII* and *TaqI*); 5,248,605 (*HpaII*); 5,312,746 (*ClaI*); 5,231,021 and 5,304,480 (*XhoI* and *XhoII*); 5,334,526 (*AluI*); 5,470,740 (*NsiI*); 5,534,428 (*SstI/SacI*); 5,202,248 (*NcoI*); 5,139,942 (*NdeI*); and 5,098,839 (*PacI*). See also Wilson, G.G., *Nucl. Acids Res.* 19:2539-2566 (1991); and Lunnen, K.D., *et al.*, *Gene* 74:25-32 (1988).

In the second form, segment *D* carries a Selectable marker. The toxic gene would eliminate transformants harboring the Vector Donor, Cointegrate, and Byproduct molecules, while the Selectable marker can be used to select for cells containing the Product and against cells harboring only the Insert Donor.

The third form selects for cells that have both segments *A* and *D* in *cis* on the same molecule, but not for cells that have both segments in *trans* on different molecules. This could be embodied by a Selectable marker that is split into two inactive fragments, one each on segments *A* and *D*.

The fragments are so arranged relative to the recombination sites that when the segments are brought together by the recombination event, they reconstitute a functional Selectable marker. For example, the recombinational event can link a promoter with a structural nucleic acid molecule (e.g., a gene), can link two fragments of a structural nucleic acid molecule, or can link nucleic acid molecules that encode a heterodimeric gene product needed for survival, or can link portions of a replicon.

**Site-specific recombinase:** is a type of recombinase which typically has at least the following four activities (or combinations thereof): (1) recognition of one or two specific nucleic acid sequences; (2) cleavage of said sequence or sequences; (3) topoisomerase activity involved in strand exchange; and (4) ligase

activity to reseal the cleaved strands of nucleic acid. See Sauer, B., *Current Opinions in Biotechnology* 5:521-527 (1994). Conservative site-specific recombination is distinguished from homologous recombination and transposition by a high degree of sequence specificity for both partners. The strand exchange mechanism involves the cleavage and rejoining of specific DNA sequences in the absence of DNA synthesis (Landy, A. (1989) *Ann. Rev. Biochem.* 58:913-949).

**Subcloning vector:** is a cloning vector comprising a circular or linear nucleic acid molecule which includes preferably an appropriate replicon. In the present invention, the subcloning vector (segment *D* in Figure 1) can also contain functional and/or regulatory elements that are desired to be incorporated into the final product to act upon or with the cloned DNA Insert (segment *A* in Figure 1). The subcloning vector can also contain a Selectable marker (preferably DNA).

**Vector:** is a nucleic acid molecule (preferably DNA) that provides a useful biological or biochemical property to an Insert. Examples include plasmids, phages, autonomously replicating sequences (ARS), centromeres, and other sequences which are able to replicate or be replicated *in vitro* or in a host cell, or to convey a desired nucleic acid segment to a desired location within a host cell. A Vector can have one or more restriction endonuclease recognition sites at which the sequences can be cut in a determinable fashion without loss of an essential biological function of the vector, and into which a nucleic acid fragment can be spliced in order to bring about its replication and cloning. Vectors can further provide primer sites, e.g., for PCR, transcriptional and/or translational initiation and/or regulation sites, recombinational signals, replicons, Selectable markers, etc. Clearly, methods of inserting a desired nucleic acid fragment which do not require the use of homologous recombination, transpositions or restriction enzymes (such as, but not limited to, UDG cloning of PCR fragments (U.S. Patent No. 5,334,575, entirely incorporated herein by reference), T:A cloning, and the like) can also be applied to clone a fragment into a cloning vector to be used according to the present invention. The cloning vector can further contain one or more selectable markers suitable for use in the identification of cells transformed with the cloning vector.

**Vector Donor:** is one of the two parental nucleic acid molecules (e.g. RNA or DNA) of the present invention which carries the DNA segments comprising the DNA vector which is to become part of the desired Product. The Vector Donor comprises a subcloning vector *D* (or it can be called the cloning vector if the Insert Donor does not already contain a cloning vector (e.g., for PCR fragments containing *attB* sites; see below)) and a segment *C* flanked by recombination sites (see Figure 1). Segments *C* and/or *D* can contain elements that contribute to selection for the desired Product daughter molecule, as described above for selection schemes. The recombination signals can be the same or different, and can be acted upon by the same or different recombinases. In addition, the Vector Donor can be linear or circular. Examples of such Vector Donor molecules include GATEWAY™ Destination Vectors, which include but are not limited to those Destination Vectors depicted in Figures 21-47 and 90-96.

**Primer:** refers to a single stranded or double stranded oligonucleotide that is extended by covalent bonding of nucleotide monomers during amplification or polymerization of a nucleic acid molecule (e.g. a DNA molecule). In a preferred aspect, a primer comprises one or more recombination sites or portions of such recombination sites. Portions of recombination sites comprise at least 2 bases (or basepairs, abbreviated herein as "bp"), at least 5-200 bases, at least 10-100 bases, at least 15-75 bases, at least 15-50 bases, at least 15-25 bases, or at least 16-25 bases, of the recombination sites of interest, as described in further detail below and in the Examples. When using portions of recombination sites, the missing portion of the recombination site may be provided as a template by the newly synthesized nucleic acid molecule. Such recombination sites may be located within and/or at one or both termini of the primer. Preferably, additional sequences are added to the primer adjacent to the recombination site(s) to enhance or improve recombination and/or to stabilize the recombination site during recombination. Such stabilization sequences may be any sequences (preferably G/C rich sequences) of any length. Preferably, such sequences range in size from 1 to about 1000 bases, 1 to about 500 bases, and 1 to about 100 bases, 1 to about 60 bases, 1 to about 25, 1 to about 10, 2 to about 10 and preferably about 4 bases.

Preferably, such sequences are greater than 1 base in length and preferably greater than 2 bases in length.

**Template:** refers to double stranded or single stranded nucleic acid molecules which are to be amplified, synthesized or sequenced. In the case of double stranded molecules, denaturation of its strands to form a first and a second strand is preferably performed before these molecules will be amplified, synthesized or sequenced, or the double stranded molecule may be used directly as a template. For single stranded templates, a primer complementary to a portion of the template is hybridized under appropriate conditions and one or more polypeptides having polymerase activity (e.g. DNA polymerases and/or reverse transcriptases) may then synthesize a nucleic acid molecule complementary to all or a portion of said template. Alternatively, for double stranded templates, one or more promoters may be used in combination with one or more polymerases to make nucleic acid molecules complementary to all or a portion of the template. The newly synthesized molecules, according to the invention, may be equal or shorter in length than the original template. Additionally, a population of nucleic acid templates may be used during synthesis or amplification to produce a population of nucleic acid molecules typically representative of the original template population.

**Adapter:** is an oligonucleotide or nucleic acid fragment or segment (preferably DNA) which comprises one or more recombination sites (or portions of such recombination sites) which in accordance with the invention can be added to a circular or linear Insert Donor molecule as well as other nucleic acid molecules described herein. When using portions of recombination sites, the missing portion may be provided by the Insert Donor molecule. Such adapters may be added at any location within a circular or linear molecule, although the adapters are preferably added at or near one or both termini of a linear molecule. Preferably, adapters are positioned to be located on both sides (flanking) a particular nucleic acid molecule of interest. In accordance with the invention, adapters may be added to nucleic acid molecules of interest by standard recombinant techniques (e.g. restriction digest and ligation). For example, adapters may be added to a circular molecule by first digesting the molecule with

an appropriate restriction enzyme, adding the adapter at the cleavage site and reforming the circular molecule which contains the adapter(s) at the site of cleavage. In other aspects, adapters may be added by homologous recombination, by integration of RNA molecules, and the like. Alternatively, adapters may be ligated directly to one or more and preferably both termini of a linear molecule thereby resulting in linear molecule(s) having adapters at one or both termini. In one aspect of the invention, adapters may be added to a population of linear molecules, (e.g. a cDNA library or genomic DNA which has been cleaved or digested) to form a population of linear molecules containing adapters at one and preferably both termini of all or substantial portion of said population.

**Adapter-Primer:** is primer molecule which comprises one or more recombination sites (or portions of such recombination sites) which in accordance with the invention can be added to a circular or linear nucleic acid molecule described herein. When using portions of recombination sites, the missing portion may be provided by a nucleic acid molecule (e.g., an adapter) of the invention. Such adapter-primers may be added at any location within a circular or linear molecule, although the adapter-primers are preferably added at or near one or both termini of a linear molecule. Examples of such adapter-primers and the use thereof in accordance with the methods of the invention are shown in Example 25 herein. Such adapter-primers may be used to add one or more recombination sites or portions thereof to circular or linear nucleic acid molecules in a variety of contexts and by a variety of techniques, including but not limited to amplification (e.g., PCR), ligation (e.g., enzymatic or chemical/synthetic ligation), recombination (e.g., homologous or non-homologous (illegitimate) recombination) and the like.

**Library:** refers to a collection of nucleic acid molecules (circular or linear). In one embodiment, a library may comprise a plurality (*i.e.*, two or more) of DNA molecules, which may or may not be from a common source organism, organ, tissue, or cell. In another embodiment, a library is representative of all or a portion or a significant portion of the DNA content of an organism (a "genomic" library), or a set of nucleic acid molecules representative of all or a portion or a significant portion of the expressed nucleic acid molecules (a cDNA library) in a

cell, tissue, organ or organism. A library may also comprise random sequences made by *de novo* synthesis, mutagenesis of one or more sequences and the like. Such libraries may or may not be contained in one or more vectors.

**Amplification:** refers to any *in vitro* method for increasing a number of copies of a nucleotide sequence with the use of a polymerase. Nucleic acid amplification results in the incorporation of nucleotides into a DNA and/or RNA molecule or primer thereby forming a new molecule complementary to a template. The formed nucleic acid molecule and its template can be used as templates to synthesize additional nucleic acid molecules. As used herein, one amplification reaction may consist of many rounds of replication. DNA amplification reactions include, for example, polymerase chain reaction (PCR). One PCR reaction may consist of 5-100 "cycles" of denaturation and synthesis of a DNA molecule.

**Oligonucleotide:** refers to a synthetic or natural molecule comprising a covalently linked sequence of nucleotides which are joined by a phosphodiester bond between the 3' position of the deoxyribose or ribose of one nucleotide and the 5' position of the deoxyribose or ribose of the adjacent nucleotide. This term may be used interchangeably herein with the terms "nucleic acid molecule" and "polynucleotide," without any of these terms necessarily indicating any particular length of the nucleic acid molecule to which the term specifically refers.

**Nucleotide:** refers to a base-sugar-phosphate combination. Nucleotides are monomeric units of a nucleic acid molecule (DNA and RNA). The term nucleotide includes ribonucleoside triphosphates ATP, UTP, CTG, GTP and deoxyribonucleoside triphosphates such as dATP, dCTP, dITP, dUTP, dGTP, dTTP, or derivatives thereof. Such derivatives include, for example, [ $\alpha$ S]dATP, 7-deaza-dGTP and 7-deaza-dATP. The term nucleotide as used herein also refers to dideoxyribonucleoside triphosphates (ddNTPs) and their derivatives. Illustrated examples of dideoxyribonucleoside triphosphates include, but are not limited to, ddATP, ddCTP, ddGTP, ddITP, and ddTTP. According to the present invention, a "nucleotide" may be unlabeled or detectably labeled by well known techniques. Detectable labels include, for example, radioactive isotopes, fluorescent labels, chemiluminescent labels, bioluminescent labels and enzyme labels.

**Hybridization:** The terms "hybridization" and "hybridizing" refers to base pairing of two complementary single-stranded nucleic acid molecules (RNA and/or DNA) to give a double stranded molecule. As used herein, two nucleic acid molecules may be hybridized, although the base pairing is not completely complementary. Accordingly, mismatched bases do not prevent hybridization of two nucleic acid molecules provided that appropriate conditions, well known in the art, are used. In some aspects, hybridization is said to be under "stringent conditions." By "stringent conditions" as used herein is meant overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Other terms used in the fields of recombinant DNA technology and molecular and cell biology as used herein will be generally understood by one of ordinary skill in the applicable arts.

### *Overview*

Two reactions constitute the recombinational cloning system of the present invention, referred to herein as the "GATEWAY™ Cloning System," as depicted generally in Figure 1. The first of these reactions, the **LR Reaction** (Figure 2), which may also be referred to interchangeably herein as the **Destination Reaction**, is the main pathway of this system. The LR Reaction is a recombination reaction between an Entry vector or clone and a Destination Vector, mediated by a cocktail of recombination proteins such as the GATEWAY™ LR Clonase™ Enzyme Mix described herein. This reaction transfers nucleic acid molecules of interest (which may be genes, cDNAs, cDNA libraries, or fragments thereof) from the Entry Clone to an Expression Vector, to create an Expression Clone.

The sites labeled L, R, B, and P are respectively the attL, attR, attB, and attP recombination sites for the bacteriophage λ recombination proteins that constitute the Clonase cocktail (referred to herein variously as "Clonase" or

“GATEWAY™ LR Clonase™ Enzyme Mix” (for recombination protein mixtures mediating attL x attR recombination reactions, as described herein) or “GATEWAY™ BP Clonase™ Enzyme Mix” (for recombination protein mixtures mediating attB x attP recombination reactions, as described herein)). The  
5 Recombinational Cloning reactions are equivalent to concerted, highly specific, cutting and ligation reactions. Viewed in this way, the recombination proteins cut to the left and right of the nucleic acid molecule of interest in the Entry Clone and ligate it into the Destination vector, creating a new Expression Clone.

The nucleic acid molecule of interest in an Expression Clone is flanked by  
10 the small attB1 and attB2 sites. The orientation and reading frame of the nucleic acid molecule of interest are maintained throughout the subcloning, because attL1 reacts only with attR1, and attL2 reacts only with attR2. Likewise, attB1 reacts only with attP1, and attB2 reacts only with attP2. Thus, the invention also relates to methods of controlled or directional cloning using the recombination sites of the  
15 invention (or portions thereof), including variants, fragments, mutants and derivatives thereof which may have altered or enhanced specificity. The invention also relates more generally to any number of recombination site partners or pairs (where each recombination site is specific for and interacts with its corresponding recombination site). Such recombination sites are preferably made by mutating or  
20 modifying the recombination site to provide any number of necessary specificities (e.g., attB1-10, attP1-10, attL1-10, attR1-10, etc.), non-limiting examples of which are described in detail in the Examples herein.

When an aliquot from the recombination reaction is transformed into host cells (e.g., *E. coli*) and spread on plates containing an appropriate selection agent,  
25 e.g., an antibiotic such as ampicillin with or without methicillin, cells that take up the desired clone form colonies. The unreacted Destination Vector does not give ampicillin-resistant colonies, even though it carries the ampicillin-resistance gene, because it contains a toxic gene, e.g., *ccdB*. Thus selection for ampicillin resistance selects for *E. coli* cells that carry the desired product, which usually  
30 comprise >90% of the colonies on the ampicillin plate.

To participate in the Recombinational (or “GATEWAY™”) Cloning Reaction, a nucleic acid molecule of interest first may be cloned into an Entry



Vector, creating an Entry Clone. Multiple options are available for creating Entry Clones, including: cloning of PCR sequences with terminal attB recombination sites into Entry Vectors; using the GATEWAY™ Cloning System recombination reaction; transfer of genes from libraries prepared in GATEWAY™ Cloning System vectors by recombination into Entry Vectors; and cloning of restriction enzyme-generated fragments and PCR fragments into Entry Vectors by standard recombinant DNA methods. These approaches are discussed in further detail herein.

A key advantage of the GATEWAY™ Cloning System is that a nucleic acid molecule of interest (or even a population of nucleic acid molecules of interest) present as an Entry Clone can be subcloned in parallel into one or more Destination Vectors in a simple reactions for anywhere from about 30 seconds to about 60 minutes (preferably about 1-60 minutes, about 1-45 minutes, about 1-30 minutes, about 2-60 minutes, about 2-45 minutes, about 2-30 minutes, about 1-2 minutes, about 30-60 minutes, about 45-60 minutes, or about 30-45 minutes). Longer reaction times (e.g., 2-24 hours, or overnight) may increase recombination efficiency, particularly where larger nucleic acid molecules are used, as described in the Examples herein. Moreover, a high percentage of the colonies obtained carry the desired Expression Clone. This process is illustrated schematically in Figure 3, which shows an advantage of the invention in which the molecule of interest can be moved simultaneously or separately into multiple Destination Vectors. In the LR Reaction, one or both of the nucleic acid molecules to be recombined may have any topology (e.g., linear, relaxed circular, nicked circular, supercoiled, etc.), although one or both are preferably linear.

The second major pathway of the GATEWAY™ Cloning System is the **BP Reaction** (Figure 4), which may also be referred to interchangeably herein as the **Entry Reaction** or the **Gateward Reaction**. The BP Reaction may recombine an Expression Clone with a Donor Plasmid (the counterpart of the byproduct in Figure 2). This reaction transfers the nucleic acid molecule of interest (which may have any of a variety of topologies, including linear, coiled, supercoiled, etc.) in the Expression Clone into an Entry Vector, to produce a new Entry Clone. Once this nucleic acid molecule of interest is cloned into an Entry

Vector, it can be transferred into new Expression Vectors, through the LR Reaction as described above. In the BP Reaction, one or both of the nucleic acid molecules to be recombined may have any topology (*e.g.*, linear, relaxed circular, nicked circular, supercoiled, etc.), although one or both are preferably linear.

5 A useful variation of the BP Reaction permits rapid cloning and expression of products of amplification (*e.g.*, PCR) or nucleic acid synthesis. Amplification (*e.g.*, PCR) products synthesized with primers containing terminal 25 bp attB sites serve as efficient substrates for the Gateway Cloning reaction. Such amplification products may be recombined with a Donor Vector to produce an Entry Clone (see 10 Figure 7). The result is an Entry Clone containing the amplification fragment. Such Entry Clones can then be recombined with Destination Vectors -- through the LR Reaction -- to yield Expression Clones of the PCR product.

Additional details of the LR Reaction are shown in Figure 5A. The GATEWAY™ LR Clonase™ Enzyme Mix that mediates this reaction contains 15 lambda recombination proteins Int (Integrase), Xis (Excisionase), and IHF (Integration Host Factor). In contrast, the GATEWAY™ BP Clonase™ Enzyme Mix, which mediates the BP Reaction (Figure 5B), comprises Int and IHF alone.

The recombination (att) sites of each vector comprise two distinct 20 segments, donated by the parental vectors. The staggered lines dividing the two portions of each att site, depicted in Figures 5A and 5B, represent the seven-base staggered cut produced by Int during the recombination reactions. This structure is seen in greater detail in Figure 6, which displays the attB recombination sequences of an Expression Clone, generated by recombination between the attL1 and attL2 sites of an Entry Clone and the attR1 and attR2 sites of a Destination 25 Vector.

The nucleic acid molecule of interest in the Expression Clone is flanked by attB sites: attB1 to the left (amino terminus) and attB2 to the right (carboxy terminus). The bases in attB1 to the left of the seven-base staggered cut produced by Int are derived from the Destination vector, and the bases to the right of the 30 staggered cut are derived from the Entry Vector (see Figure 6). Note that the sequence is displayed in triplets corresponding to an open reading frame. If the reading frame of the nucleic acid molecule of interest cloned in the Entry Vector

is in phase with the reading frame shown for attB1, amino-terminal protein fusions can be made between the nucleic acid molecule of interest and any GATEWAY™ Cloning System Destination Vector encoding an amino-terminal fusion domain. Entry Vectors and Destination Vectors that enable cloning in all three reading frames are described in more detail herein, particularly in the Examples.

The LR Reaction allows the transfer of a desired nucleic acid molecule of interest into new Expression Vectors by recombining a Entry Clone with various Destination Vectors. To participate in the LR or Destination Reaction, however, a nucleic acid molecule of interest preferably is first converted to a Entry Clone. Entry Clones can be made in a number of ways, as shown in Figure 7.

One approach is to clone the nucleic acid molecule of interest into one or more of the Entry Vectors, using standard recombinant DNA methods, with restriction enzymes and ligase. The starting DNA fragment can be generated by restriction enzyme digestion or as a PCR product. The fragment is cloned between the attL1 and attL2 recombination sites in the Entry Vector. Note that a toxic or "death" gene (*e.g.*, *ccdB*), provided to minimize background colonies from incompletely digested Entry Vector, must be excised and replaced by the nucleic acid molecule of interest.

A second approach to making an Entry Clone (Figure 7) is to make a library (genomic or cDNA) in an Entry Vector, as described in detail herein. Such libraries may then be transferred into Destination Vectors for expression screening, for example in appropriate host cells such as yeast cells or mammalian cells.

A third approach to making Entry Clones (Figure 7) is to use Expression Clones obtained from cDNA molecules or libraries prepared in Expression Vectors. Such cDNAs or libraries, flanked by attB sites, can be introduced into a Entry Vector by recombination with a Donor Vector via the BP Reaction. If desired, an entire Expression Clone library can be transferred into the Entry Vector through the BP Reaction. Expression Clone cDNA libraries may also be constructed in a variety of prokaryotic and eukaryotic GATEWAY™-modified vectors (*e.g.*, the pEXP501 Expression Vector (see Figure 48), and 2-hybrid and

attB library vectors), as described in detail herein, particularly in the Examples below.

A fourth, and potentially most versatile, approach to making an Entry Clone (Figure 7) is to introduce a sequence for a nucleic acid molecule of interest into an Entry Vector by amplification (*e.g.*, PCR) fragment cloning. This method is diagramed in Figure 8. The DNA sequence first is amplified (for example, with PCR) as outlined in detail below and in the Examples herein, using primers containing one or more bp, two or more bp, three or more bp, four or more bp, five or more bp, preferably six or more bp, more preferably 6-25 bp (particularly 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25) bp of the attB nucleotide sequences (such as, but not limited to, those depicted in Figure 9), and optionally one or more, two or more, three or more, four or more, and most preferably four or five or more additional terminal nucleotide bases which preferably are guanines. The PCR product then may be converted to a Entry Clone by performing a BP Reaction, in which the attB-PCR product recombines with a Donor Vector containing one or more attP sites. Details of this approach and protocols for PCR fragment subcloning are provided in Examples 8 and 21-25.

A variety of Entry Clones may be produced by these methods, providing a wide array of cloning options; a number of specific Entry Vectors are also available commercially from Life Technologies, Inc. (Rockville, MD). The Examples herein provide a more in-depth description of selected Entry Vectors and details of their cloning sites. Choosing the optimal Entry Vector for a particular application is discussed in Example 4.

Entry Vectors and Destination Vectors should be constructed so that the amino-terminal region of a nucleic acid molecule of interest (*e.g.*, a gene, cDNA library or insert, or fragment thereof) will be positioned next to the attL1 site. Entry Vectors preferably contain the *rrnB* transcriptional terminator upstream of the attL1 site. This sequence ensures that expression of cloned nucleic acid molecules of interest is reliably "off" in *E. coli*, so that even toxic genes can be successfully cloned. Thus, Entry Clones may be designed to be transcriptionally silent. Note also that Entry Vectors, and hence Entry Clones, may contain the kanamycin antibiotic resistance (*kan<sup>r</sup>*) gene to facilitate selection of host cells

containing Entry Clones after transformation. In certain applications, however, Entry Clones may contain other selection markers, including but not limited to a gentamycin resistance (*gen<sup>r</sup>*) or tetracycline resistance (*tet<sup>r</sup>*) gene, to facilitate selection of host cells containing Entry Clones after transformation.

5           Once a nucleic acid molecule of interest has been cloned into an Entry Vector, it may be moved into a Destination Vector. The upper right portion of Figure 5A shows a schematic of a Destination Vector. The thick arrow represents some function (often transcription or translation) that will act on the nucleic acid molecule of interest in the clone. During the recombination reaction, the region  
10           between the attR1 and attR2 sites, including a toxic or "death" gene (*e.g.*, *ccdB*), is replaced by the DNA segment from the Entry Clone. Selection for recombinants that have acquired the ampicillin resistance (*amp<sup>r</sup>*) gene (carried on the Destination Vector) and that have also lost the death gene ensures that a high percentage (usually >90%) of the resulting colonies will contain the correct insert.

15           To move a nucleic acid molecule of interest into a Destination Vector, the Destination Vector is mixed with the Entry Clone comprising the desired nucleic acid molecule of interest, a cocktail of recombination proteins (*e.g.*, GATEWAY™ LR Clonase™ Enzyme Mix) is added, the mixture is incubated (preferably at about 25°C for about 60 minutes, or longer under certain  
20           circumstances, *e.g.* for transfer of large nucleic acid molecules, as described below) and any standard host cell (including bacterial cells such as *E. coli*; animal cells such as insect cells, mammalian cells, nematode cells and the like; plant cells; and yeast cells) strain is transformed with the reaction mixture. The host cell used will be determined by the desired selection (*e.g.*, *E. coli* DB3.1, available  
25           commercially from Life Technologies, Inc., allows survival of clones containing the *ccdB* death gene, and thus can be used to select for cointegrate molecules -- *i.e.*, molecules that are hybrids between the Entry Clone and Destination Vector). The Examples below provide further details and protocols for use of Entry and Destination Vectors in transferring nucleic acid molecules of interest and  
30           expressing RNAs or polypeptides encoded by these nucleic acid molecules in a variety of host cells.

The cloning system of the invention therefore offers multiple advantages:

- Once a nucleic acid molecule of interest is cloned into the GATEWAY™ Cloning System, it can be moved into and out of other vectors with complete fidelity of reading frame and orientation. That is, since the reactions proceed whereby attL1 on the Entry Clone recombines with attR1 on the Destination Vector, the directionality of the nucleic acid molecule of interest is maintained or may be controlled upon transfer from the Entry Clone into the Destination Vector. Hence, the GATEWAY™ Cloning System provides a powerful and easy method of directional cloning of nucleic acid molecule of interest.
- One-step cloning or subcloning: Mix the Entry Clone and the Destination Vector with Clonase, incubate, and transform.
- Clone PCR products readily by *in vitro* recombination, by adding attB sites to PCR primers. Then directly transfer these Entry Clones into Destination Vectors. This process may also be carried out in one step (see Examples below).
- Powerful selections give high reliability: >90% ( and often >99%) of the colonies contain the desired DNA in its new vector.
- One-step conversion of existing standard vectors into GATEWAY™ Cloning System vectors.
- Ideal for large vectors or those with few cloning sites.
- Recombination sites are short (25 bp), and may be engineered to contain no stop codons or secondary structures.
- Reactions may be automated, for high-throughput applications (*e.g.*, for diagnostic purposes or for therapeutic candidate screening).
- The reactions are economical: 0.3 µg of each DNA; no restriction enzymes, phosphatase, ligase, or gel purification. Reactions work well with miniprep DNA.
- Transfer multiple clones, and even libraries, into one or more Destination Vectors, in a single experiment.
- A variety of Destination Vectors may be produced, for applications including, but not limited to:

- Protein expression in *E. coli*: native proteins; fusion proteins with GST, His6, thioredoxin, etc., for purification, or one or more epitope tags; any promoter useful in expressing proteins in *E. coli* may be used, such as ptrc,  $\lambda P_L$ , and T7 promoters.
- Protein expression in eukaryotic cells: CMV promoter, baculovirus (with or without His6 tag), Semliki Forest virus, Tet regulation.
- DNA sequencing (all *lac* primers), RNA probes, phagemids (both strands)
- A variety of Entry Vectors (for recombinational cloning entry by standard recombinant DNA methods) may be produced:
  - Strong transcription stop just upstream, for genes toxic to *E. coli*.
  - Three reading frames.
  - With or without TEV protease cleavage site.
  - Motifs for prokaryotic and / or eukaryotic translation.
  - Compatible with commercial cDNA libraries.
- Expression Clone cDNA (*attB*) libraries, for expression screening, including 2-hybrid libraries and phage display libraries, may also be constructed.

### ***Recombination Site Sequences***

In one aspect, the invention relates to nucleic acid molecules, which may or may not be isolated nucleic acid molecules, comprising one or more nucleotide sequences encoding one or more recombination sites or portions thereof. In particular, this aspect of the invention relates to such nucleic acid molecules comprising one or more nucleotide sequences encoding *attB*, *attP*, *attL*, or *attR*, or portions of these recombination site sequences. The invention also relates to mutants, derivatives, and fragments of such nucleic acid molecules. Unless otherwise indicated, all nucleotide sequences that may have been determined by sequencing a DNA molecule herein were determined using manual or automated DNA sequencing, such as dideoxy sequencing, according to methods that are routine to one of ordinary skill in the art (Sanger, F., and Coulson, A.R., *J. Mol. Biol.* 94:444-448 (1975); Sanger, F., *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)). All amino acid sequences of polypeptides encoded by DNA

molecules determined herein were predicted by conceptual translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by these approaches, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by such methods are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Unless otherwise indicated, each "nucleotide sequence" set forth herein is presented as a sequence of deoxyribonucleotides (abbreviated A, G, C and T). However, by "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U). Thus, the invention relates to sequences of the invention in the form of DNA or RNA molecules, or hybrid DNA/RNA molecules, and their corresponding complementary DNA, RNA, or DNA/RNA strands.

In a first such aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attB1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attB1* nucleotide sequence having the sequence set forth in Figure 9, such as: ACAAGTTTGTACAAAAAAGCAGGCT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attB1*, or mutants, fragments, variants or derivatives thereof. As one of ordinary skill will appreciate, however, certain mutations, insertions, or deletions of one or more bases in the *attB1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional



integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attB1* sequence are encompassed within the scope of the invention.

In a related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attB2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attB2* nucleotide sequence having the sequence set forth in Figure 9, such as: ACCCAGCTTTCTTGTACAAAGTGGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attB2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attB2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attB2* sequence are encompassed within the scope of the invention.

A recombinant host cell comprising a nucleic acid molecule containing *attB1* and *attB2* sites (the vector pEXP501, also known as pCMVSPORT6; see Figure 48), *E. coli* DB3.1(pCMVSPORT6), was deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit No. NRRL B-30108. The *attB1* and *attB2* sites within the deposited nucleic acid molecule are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attP1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attP1* nucleotide sequence having the sequence set forth in Figure 9, such as: TACAGGTCACCTAATACCATCTAAGTAGTTGATTCATAGTGA-CTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTAT-GCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTT-TCTCGTTCAGCTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTG-CTCATCAATTTGTTGCAACGAACAGGTCACCTATCAGTCAAAATAA-

AATCATTATTTG, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attP1*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attP1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attP1* sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attP2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attP2* nucleotide sequence having the sequence set forth in Figure 9, such as: CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTG-CAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTT-GTACAAGAAAGCTGAACGAGAAACGTAAAATGATA-TAAATATCAATATATTAATTAGATTTTGCATAAAAAACAG-ACTACATAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATGGTATTAGTGACCTGTA, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attP2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attP2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attP2* sequence are encompassed within the scope of the invention.

A recombinant host cell comprising a nucleic acid molecule (the *attP* vector pDONR201, also known as pENTR21-*attPkan* or p*AttPkan*; see Figure 49) containing *attP1* and *attP2* sites, *E. coli* DB3.1(p*AttPkan*) (also called *E. coli* DB3.1(pAHK*an*)), was deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit No. NRRL B-30099. The *attP1* and *attP2* sites within the deposited nucleic acid molecule are contained in nucleic acid

cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attR1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attR1* nucleotide sequence having the sequence set forth in Figure 9, such as: ACAAGTTTGTACAAAAAGCTGAACGAG-AAACGTAATAATGATATAAATATCAATATATTAAATTAGATTTTGCAT-AAAAACAGACTACATAATACTGTAAACACAACATATCCAGTCA-CTATG, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attR1*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attR1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attR1* sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attR2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attR2* nucleotide sequence having the sequence set forth in Figure 9, such as: GCAGGTCGACCATAGTGACTGGATAT-GTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTA-ATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTT-TCTTGTACAAAGTGGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attR2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attR2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attR2* sequence are encompassed within the scope of the invention.

Recombinant host cell strains containing attR1 sites apposed to cloning sites in reading frame A, reading frame B, and reading frame C, *E. coli* DB3.1(pEZC15101) (reading frame A; see Figure 64A), *E. coli* DB3.1(pEZC15102) (reading frame B; see Figure 64B), and *E. coli* DB3.1(pEZC15103) (reading frame C; see Figure 64C), and containing corresponding attR2 sites, were deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit Nos. NRRL B-30103, NRRL B-30104, and NRRL B-30105, respectively. The attR1 and attR2 sites within the deposited nucleic acid molecules are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding attL1, or mutants, fragments, variants and derivatives thereof. Such nucleic acid molecules may comprise an attL1 nucleotide sequence having the sequence set forth in Figure 9, such as: CAA ATA ATG ATT TTA TTT TGA CTG ATA GTG ACC TGT TCG TTG CAA CAA ATT GAT AAG CAA TGC TTT TTT ATA ATG CCA ACT TTG TAC AAA AAA GCA GGC T, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for attL1, or mutants, fragments, variants or derivatives thereof. As noted above for attB1, certain mutations, insertions, or deletions of one or more bases in the attL1 sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the attL1 sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding attL2, or mutants, fragments, variants and derivatives thereof. Such nucleic acid molecules may comprise an attL2 nucleotide sequence having the sequence set forth in Figure 9, such as: C AAA TAA TGA TTT TAT TTT GAC TGA TAG TGA CCT GTT CGT TGC AAC AAA TTG ATA AGC AAT GCT TTC TTA TAA TGC CAA

CTT TGT ACA AGA AAG CTG GGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attL2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attL2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attL2* sequence are encompassed within the scope of the invention.

Recombinant host cell strains containing *attL1* sites apposed to cloning sites in reading frame A, reading frame B, and reading frame C, *E. coli* DB3.1(pENTR1A) (reading frame A; see Figure 10), *E. coli* DB3.1(pENTR2B) (reading frame B; see Figure 11), and *E. coli* DB3.1(pENTR3C) (reading frame C; see Figure 12), and containing corresponding *attL2* sites, were deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit Nos. NRRL B-30100, NRRL B-30101, and NRRL B-30102, respectively. The *attL1* and *attL2* sites within the deposited nucleic acid molecules are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

Each of the recombination site sequences described herein or portions thereof, or the nucleotide sequence cassettes contained in the deposited clones, may be cloned or inserted into a vector of interest (for example, using the recombinational cloning methods described herein and/or standard restriction cloning techniques that are routine in the art) to generate, for example, Entry Vectors or Destination Vectors which may be used to transfer a desired segment of a nucleic acid molecule of interest (e.g., a gene, cDNA molecule, or cDNA library) into a desired vector or into a host cell.

Using the information provided herein, such as the nucleotide sequences for the recombination site sequences described herein, an isolated nucleic acid molecule of the present invention encoding one or more recombination sites or portions thereof may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Preferred such

methods include PCR-based cloning methods, such as reverse transcriptase-PCR (RT-PCR) using primers such as those described herein and in the Examples below. Alternatively, vectors comprising the cassettes containing the recombination site sequences described herein are available commercially from Life Technologies, Inc. (Rockville, MD).

The invention is also directed to nucleic acid molecules comprising one or more of the recombination site sequences or portions thereof and one or more additional nucleotide sequences, which may encode functional or structural sites such as one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (which may be promoters, enhancers, repressors, and the like), one or more translational signals (*e.g.*, secretion signal sequences), one or more origins of replication, one or more fusion partner peptides (particularly glutathione S-transferase (GST), hexahistidine (His<sub>6</sub>), and thioredoxin (Trx)), one or more selection markers or modules, one or more nucleotide sequences encoding localization signals such as nuclear localization signals or secretion signals, one or more origins of replication, one or more protease cleavage sites, one or more genes or portions of genes encoding a protein or polypeptide of interest, and one or more 5' polynucleotide extensions (particularly an extension of guanine residues ranging in length from about 1 to about 20, from about 2 to about 15, from about 3 to about 10, from about 4 to about 10, and most preferably an extension of 4 or 5 guanine residues at the 5' end of the recombination site nucleotide sequence. The one or more additional functional or structural sequences may or may not flank one or more of the recombination site sequences contained on the nucleic acid molecules of the invention.

In some nucleic acid molecules of the invention, the one or more nucleotide sequences encoding one or more additional functional or structural sites may be operably linked to the nucleotide sequence encoding the recombination site. For example, certain nucleic acid molecules of the invention may have a promoter sequence operably linked to a nucleotide sequence encoding a recombination site or portion thereof of the invention, such as a T7 promoter, a phage lambda PL

promoter, an *E. coli lac*, *trp* or *tac* promoter, and other suitable promoters which will be familiar to the skilled artisan.

Nucleic acid molecules of the present invention, which may be isolated nucleic acid molecules, may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically, or in the form of DNA-RNA hybrids. The nucleic acid molecules of the invention may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand. The nucleic acid molecules of the invention may also have a number of topologies, including linear, circular, coiled, or supercoiled.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells, and those DNA molecules purified (partially or substantially) from a solution whether produced by recombinant DNA or synthetic chemistry techniques. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention.

The present invention further relates to mutants, fragments, variants and derivatives of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of one or more recombination sites. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (*see* Lewin, B., ed., *Genes II*, , John Wiley & Sons, New York (1985)). Non-naturally occurring variants may be produced using art-known mutagenesis techniques, such as those described hereinbelow.

Such variants include those produced by nucleotide substitutions, deletions or additions or portions thereof, or combinations thereof. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding

regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the encoded polypeptide(s) or portions thereof, and which also do not substantially alter the reactivities of the recombination site nucleic acid sequences in recombination reactions. Also especially preferred in this regard are conservative substitutions.

Particularly preferred mutants, fragments, variants, and derivatives of the nucleic acid molecules of the invention include, but are not limited to, insertions, deletions or substitutions of one or more nucleotide bases within the 15 bp core region (GCTTTTTTATACTAA) which is identical in all four wildtype lambda *att* sites, *attB*, *attP*, *attL* and *attR* (see U.S. Application Nos. 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, which describes the core region in further detail, and the disclosures of which are incorporated herein by reference in their entireties). Analogously, the core regions in *attB1*, *attP1*, *attL1* and *attR1* are identical to one another, as are the core regions in *attB2*, *attP2*, *attL2* and *attR2*. Particularly preferred in this regard are nucleic acid molecules comprising insertions, deletions or substitutions of one or more nucleotides within the seven bp overlap region (TTTATAC, which is defined by the cut sites for the integrase protein and is the region where strand exchange takes place) that occurs within this 15 bp core region (GCTTTTTTTTTATACTAA). Examples of such preferred mutants, fragments, variants and derivatives according to this aspect of the invention include, but are not limited to, nucleic acid molecules in which the thymine at position 1 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the thymine at position 2 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the thymine at position 3 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the adenine at position 4 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or thymine; in which the thymine at position 5 of the seven bp overlap region has been deleted or substituted with a



guanine, cytosine, or adenine; in which the adenine at position 6 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or thymine; and in which the cytosine at position 7 of the seven bp overlap region has been deleted or substituted with a guanine, thymine, or adenine; or any combination of one or more such deletions and/or substitutions within this seven bp overlap region. As described in detail in Example 21 herein, mutants of the nucleic acid molecules of the invention in which substitutions have been made within the first three positions of the seven bp overlap (TTTATAC) have been found in the present invention to strongly affect the specificity of recombination, mutant nucleic acid molecules in which substitutions have been made in the last four positions (TTTATAC) only partially alter recombination specificity, and mutant nucleic acid molecules comprising nucleotide substitutions outside of the seven bp overlap, but elsewhere within the 15 bp core region, do not affect specificity of recombination but do influence the efficiency of recombination.

Hence, in an additional aspect, the present invention is also directed to nucleic acid molecules comprising one or more recombination site nucleotide sequences that affect recombination specificity, particularly one or more nucleotide sequences that may correspond substantially to the seven base pair overlap within the 15 bp core region, having one or more mutations that affect recombination specificity. Particularly preferred such molecules may comprise a consensus sequence (described in detail in Example 21 herein) such as NNNATAC, wherein "N" refers to any nucleotide (*i.e.*, may be A, G, T/U or C), with the proviso that if one of the first three nucleotides in the consensus sequence is a T/U, then at least one of the other two of the first three nucleotides is not a T/U.

In a related aspect, the present invention is also directed to nucleic acid molecules comprising one or more recombination site nucleotide sequences that enhance recombination efficiency, particularly one or more nucleotide sequences that may correspond substantially to the core region and having one or more mutations that enhance recombination efficiency. By sequences or mutations that "enhance recombination efficiency" is meant a sequence or mutation in a recombination site, preferably in the core region (*e.g.*, the 15 bp core region of *att* recombination sites), that results in an increase in cloning efficiency (typically

measured by determining successful cloning of a test sequence, e.g., by determining CFU/ml for a given cloning mixture) when recombining molecules comprising the mutated sequence or core region as compared to molecules that do not comprise the mutated sequence or core region (e.g., those comprising a wildtype recombination site core region sequence). More specifically, whether or not a given sequence or mutation enhances recombination efficiency may be determined using the sequence or mutation in recombinational cloning as described herein, and determining whether the sequence or mutation provides enhanced recombinational cloning efficiency when compared to a non-mutated (e.g., wildtype) sequence. Methods of determining preferred cloning efficiency-enhancing mutations for a number of recombination sites, particularly for *att* recombination sites, are described herein, for example in Examples 22-25. Examples of preferred such mutant recombination sites include but are not limited to the *attL* consensus core sequence of caacttntntnnnannaagttg (wherein "n" represents any nucleotide), for example the *attL5* sequence agcctgctttattataactaagttggcatta and the *attL6* sequence agcctgcttttttatattaagttggcatta; the *attB1.6* sequence ggggacaactttgtacaaaaagttggct; the *attB2.2* sequence ggggacaactttgtacaagaaagctgggt; and the *attB2.10* sequence ggggacaactttgtacaagaaagttgggt. Those of skill in the art will appreciate that, in addition to the core region, other portions of the *att* site may affect the efficiency of recombination. There are five so-called arm binding sites for the integrase protein in the bacteriophage lambda *attP* site, two in *attR* (P1 and P2), and three in *attL* (P'1, P'2 and P'3). Compared to the core binding sites, the integrase protein binds to arm sites with high affinity and interacts with core and arm sites through two different domains of the protein. As with the core binding site a consensus sequence for the arm binding site consisting of C/AAGTCACTAT has been inferred from sequence comparison of the five arm binding sites and seven non-*att* sites (Ross and Landy, *Proc. Natl. Acad. Sci. USA* 79:7724-7728 (1982)). Each arm site has been mutated and tested for its effect in the excision and integration reactions (Numrych *et al.*, *Nucl. Acids Res.* 18:3953 (1990)). Hence, specific sites are utilized in each reaction in different ways, namely, the P1 and P'3

sites are essential for the integration reaction whereas the other three sites are dispensable to the integration reaction to varying degrees. Similarly, the P2, P'1 and P'2 sites are most important for the excision reaction, whereas P1 and P'3 are completely dispensable. Interestingly, when P2 is mutated the integration reaction occurs more efficiently than with the wild type attP site. Similarly, when P1 and P'3 are mutated the excision reaction occurs more efficiently. The stimulatory effect of mutating integrase arm binding sites can be explained by removing sites that compete or inhibit a specific recombination pathway or that function in a reaction that converts products back to starting substrates. In fact there is evidence for an XIS-independent LR reaction (Abremski and Gottesman, *J. Mol. Biol.* 153:67-78 (1981)). Thus, in addition to modifications in the core region of the att site, the present invention contemplates the use of att sites containing one or more modifications in the integrase arm-type binding sites. In some preferred embodiments, one or more mutations may be introduced into one or more of the P1, P'1, P2, P'2 and P'3 sites. In some preferred embodiments, multiple mutations may be introduced into one or more of these sites. Preferred such mutations include those which increase the recombination *in vitro*. For example, in some embodiments mutations may be introduced into the arm-type binding sites such that integrative recombination, corresponding to the BP reaction, is enhanced. In other embodiments, mutations may be introduced into the arm-type binding sites such that excisive recombination, corresponding to the LR reaction, is enhanced. Of course, based on the guidance contained herein, particularly in the construction and evaluation of effects of mutated recombination sites upon recombinational specificity and efficiency, analogous mutated or engineered sequences may be produced for other recombination sites described herein (including but not limited to *lox*, FRT, and the like) and used in accordance with the invention. For example, much like the mutagenesis strategy used to select core binding sites that enhance recombination efficiency, similar strategies can be employed to select changes in the arms of attP, attL and attR, and in analogous sequences in other recombination sites such as *lox*, FRT and the like, that enhance recombination efficiency. Hence, the construction and evaluation of such mutants is well within the abilities of those of ordinary skill in the art without undue experimentation.

One suitable methodology for preparing and evaluating such mutations is found in Numrych, *et al.*, (1990) *Nucleic Acids Research* 18(13): 3953-3959.

Other mutant sequences and nucleic acid molecules that may be suitable to enhance recombination efficiency will be apparent from the description herein, or may be easily determined by one of ordinary skill using only routine experimentation in molecular biology in view of the description herein and information that is readily available in the art

Since the genetic code is well known in the art, it is also routine for one of ordinary skill in the art to produce degenerate variants of the nucleic acid molecules described herein without undue experimentation. Hence, nucleic acid molecules comprising degenerate variants of nucleic acid sequences encoding the recombination sites described herein are also encompassed within the scope of the invention.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 50% identical, at least 60% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to the nucleotide sequences of the seven bp overlap region within the 15 bp core region of the recombination sites described herein, or the nucleotide sequences of *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* as set forth in Figure 9 (or portions thereof), or a nucleotide sequence complementary to any of these nucleotide sequences, or fragments, variants, mutants, and derivatives thereof.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a particular recombination site or portion thereof is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations (*e.g.*, insertions, substitutions, or deletions) per each 100 nucleotides of the reference nucleotide sequence encoding the recombination site. For example, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference *attB1* nucleotide sequence, up to 5% of the nucleotides in the *attB1* reference sequence may be

deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the *attB1* reference sequence may be inserted into the *attB1* reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or  
5 anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical  
10 to, for instance, a given recombination site nucleotide sequence or portion thereof can be determined conventionally using known computer programs such as DNAsis software (Hitachi Software, San Bruno, California) for initial sequence alignment followed by ESEE version 3.0 DNA/protein sequence software (cabot@trog.mbb.sfu.ca) for multiple sequence alignments. Alternatively, such  
15 determinations may be accomplished using the BESTFIT program (Wisconsin Sequence Analysis Package, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711), which employs a local homology algorithm (Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981)) to find the best segment of homology between two sequences. When  
20 using DNAsis, ESEE, BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number  
25 of nucleotides in the reference sequence are allowed.

The present invention is directed to nucleic acid molecules at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* nucleotide sequences as set forth in Figure 9, or to the nucleotide sequence of the deposited clones, irrespective of  
30 whether they encode particular functional polypeptides. This is because even where a particular nucleic acid molecule does not encode a particular functional polypeptide, one of skill in the art would still know how to use the nucleic acid

molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer.

5 Mutations can also be introduced into the recombination site nucleotide sequences for enhancing site specific recombination or altering the specificities of the reactants, etc. Such mutations include, but are not limited to: recombination sites without translation stop codons that allow fusion proteins to be encoded; recombination sites recognized by the same proteins but differing in base sequence such that they react largely or exclusively with their homologous partners allowing multiple reactions to be contemplated; and mutations that prevent hairpin formation of recombination sites. Which particular reactions take place can be specified by which particular partners are present in the reaction mixture.

10 There are well known procedures for introducing specific mutations into nucleic acid sequences. A number of these are described in Ausubel, F.M. *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1989-1996). Mutations can be designed into oligonucleotides, which can be used to modify existing cloned sequences, or in amplification reactions. Random mutagenesis can also be employed if appropriate selection methods are available to isolate the desired mutant DNA or RNA. The presence of the desired mutations can be confirmed by sequencing the nucleic acid by well known methods.

20 The following non-limiting methods can be used to modify or mutate a given nucleic acid molecule encoding a particular recombination site to provide mutated sites that can be used in the present invention:

- 25 1. By recombination of two parental DNA sequences by site-specific (e.g. attL and attR to give attP) or other (e.g. homologous) recombination mechanisms where the parental DNA segments contain one or more base alterations resulting in the final mutated nucleic acid molecule;
2. By mutation or mutagenesis (site-specific, PCR, random, spontaneous, etc) directly of the desired nucleic acid molecule;
- 30 3. By mutagenesis (site-specific, PCR, random, spontaneous, etc) of parental DNA sequences, which are recombined to generate a desired nucleic acid molecule;

4. By reverse transcription of an RNA encoding the desired core sequence;  
and
5. By *de novo* synthesis (chemical synthesis) of a sequence having the desired  
base changes, or random base changes followed by sequencing or  
functional analysis according to methods that are routine in the art.

The functionality of the mutant recombination sites can be demonstrated in ways that depend on the particular characteristic that is desired. For example, the lack of translation stop codons in a recombination site can be demonstrated by expressing the appropriate fusion proteins. Specificity of recombination between homologous partners can be demonstrated by introducing the appropriate molecules into *in vitro* reactions, and assaying for recombination products as described herein or known in the art. Other desired mutations in recombination sites might include the presence or absence of restriction sites, translation or transcription start signals, protein binding sites, particular coding sequences, and other known functionalities of nucleic acid base sequences. Genetic selection schemes for particular functional attributes in the recombination sites can be used according to known method steps. For example, the modification of sites to provide (from a pair of sites that do not interact) partners that do interact could be achieved by requiring deletion, via recombination between the sites, of a DNA sequence encoding a toxic substance. Similarly, selection for sites that remove translation stop sequences, the presence or absence of protein binding sites, etc., can be easily devised by those skilled in the art.

Accordingly, the present invention also provides a nucleic acid molecule, comprising at least one DNA segment having at least one, and preferably at least two, engineered recombination site nucleotide sequences of the invention flanking a selectable marker and/or a desired DNA segment, wherein at least one of said recombination site nucleotide sequences has at least one engineered mutation that enhances recombination *in vitro* in the formation of a Cointegrate DNA or a Product DNA. Such engineered mutations may be in the core sequence of the recombination site nucleotide sequence of the invention; *see* U.S. Application Nos. 08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed

October 23, 1998, the disclosures of which are all incorporated herein by reference in their entireties.

While in the preferred embodiment the recombination sites differ in sequence and do not interact with each other, it is recognized that sites comprising the same sequence, which may interact with each other, can be manipulated or engineered to inhibit recombination with each other. Such conceptions are considered and incorporated herein. For example, a protein binding site (*e.g.*, an antibody-binding site, a histone-binding site, an enzyme-binding site, or a binding site for any nucleic acid molecule-binding protein) can be engineered adjacent to one of the sites. In the presence of the protein that recognizes the engineered site, the recombinase fails to access the site and another recombination site in the nucleic acid molecule is therefore used preferentially. In the cointegrate this site can no longer react since it has been changed, *e.g.*, from attB to attL. During or upon resolution of the cointegrate, the protein can be inactivated (*e.g.*, by antibody, heat or a change of buffer) and the second site can undergo recombination.

The nucleic acid molecules of the invention can have at least one mutation that confers at least one enhancement of said recombination, said enhancement selected from the group consisting of substantially (i) favoring integration; (ii) favoring recombination; (iii) relieving the requirement for host factors; (iv) increasing the efficiency of said Cointegrate DNA or Product DNA formation; (v) increasing the specificity of said Cointegrate DNA or Product DNA formation; and (vi) adding or deleting protein binding sites.

In other embodiments, the nucleic acid molecules of the invention may be PCR primer molecules, which comprise one or more of the recombination site sequences described herein or portions thereof, particularly those shown in Figure 9 (or sequences complementary to those shown in Figure 9), or mutants, fragments, variants or derivatives thereof, attached at the 3' end to a target-specific template sequence which specifically interacts with a target nucleic acid molecule which is to be amplified. Primer molecules according to this aspect of the invention may further comprise one or more, (*e.g.*, 1, 2, 3, 4, 5, 10, 20, 25, 50, 100, 500, 1000, or more) additional bases at their 5' ends, and preferably comprise one or more (particularly four or five) additional bases, which are preferably



guanines, at their 5' ends, to increase the efficiency of the amplification products incorporating the primer molecules in the recombinational cloning system of the invention. Such nucleic acid molecules and primers are described in detail in the examples herein, particularly in Examples 22-25.

Certain primers of the invention may comprise one or more nucleotide deletions in the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* sequences as set forth in Figure 9. In one such aspect, for example, *attB2* primers may be constructed in which one or more of the first four nucleotides at the 5' end of the *attB2* sequence shown in Figure 9 have been deleted. Primers according to this aspect of the invention may therefore have the sequence:

(*attB2*(-1)): CCCAGCTTCTTGTACAAAGTGGTnnnnnnnnnnnn . . . n

(*attB2*(-2)): CCAGCTTCTTGTACAAAGTGGTnnnnnnnnnnnn . . . n

(*attB2*(-3)): CAGCTTCTTGTACAAAGTGGTnnnnnnnnnnnn . . . n

(*attB2*(-4)): AGCTTCTTGTACAAAGTGGTnnnnnnnnnnnn . . . n,

wherein "nnnnnnnnnnnn . . . n" at the 3' end of the primer represents a target-specific sequence of any length, for example from one base up to all of the bases of a target nucleic acid molecule (e.g., a gene) or a portion thereof, the sequence and length which will depend upon the identity of the target nucleic acid molecule which is to be amplified.

The primer nucleic acid molecules according to this aspect of the invention may be produced synthetically by attaching the recombination site sequences depicted in Figure 9, or portions thereof, to the 5' end of a standard PCR target-specific primer according to methods that are well-known in the art. Alternatively, additional primer nucleic acid molecules of the invention may be produced synthetically by adding one or more nucleotide bases, which preferably correspond to one or more, preferably five or more, and more preferably six or more, contiguous nucleotides of the *att* nucleotide sequences described herein (see, e.g., Example 20 herein; see also U.S. Application Nos. 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, the disclosures of which are all incorporated herein by reference in their entireties), to the 5' end of a standard PCR target-specific primer according to methods that are well-known in the art, to provide

primers having the specific nucleotide sequences described herein. As noted above, primer nucleic acid molecules according to this aspect of the invention may also optionally comprise one, two, three, four, five, or more additional nucleotide bases at their 5' ends, and preferably will comprise four or five guanines at their 5' ends. In one particularly preferred such aspect, the primer nucleic acid molecules of the invention may comprise one or more, preferably five or more, more preferably six or more, still more preferably 6-18 or 6-25, and most preferably 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25, contiguous nucleotides or bp of the *attB1* or *attB2* nucleotide sequences depicted in Figure 9 (or nucleotides complementary thereto), linked to the 5' end of a target-specific (*e.g.*, a gene-specific) primer molecule. Primer nucleic acid molecules according to this aspect of the invention include, but are not limited to, *attB1*- and *attB2*-derived primer nucleic acid molecules having the following nucleotide sequences:

ACAAGTTTGTACAAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
ACCACTTTGTACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
TGTACAAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
TGTACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
ACAAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
ACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
AAAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
AGAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
AAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
GAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
AAAGCAGGCT-nnnnnnnnnnnnn . . . n  
AAAGCTGGGT-nnnnnnnnnnnnn . . . n  
AAGCAGGCT-nnnnnnnnnnnnn . . . n  
AAGCTGGGT-nnnnnnnnnnnnn . . . n  
AGCAGGCT-nnnnnnnnnnnnn . . . n  
AGCTGGGT-nnnnnnnnnnnnn . . . n  
GCAGGCT-nnnnnnnnnnnnn . . . n  
GCTGGGT-nnnnnnnnnnnnn . . . n

CAGGCT-nnnnnnnnnnnnn . . . n

CTGGGT-nnnnnnnnnnnnn . . . n,

wherein "nnnnnnnnnnnn . . . n" at the 3' end of the primer represents a target-specific sequence of any length, for example from one base up to all of the bases of a target nucleic acid molecule (e.g., a gene) or a portion thereof, the sequence and length which will depend upon the identity of the target nucleic acid molecule which is to be amplified.

Of course, it will be apparent to one of ordinary skill from the teachings contained herein that additional primer nucleic acid molecules analogous to those specifically described herein may be produced using one or more, preferably five or more, more preferably six or more, still more preferably ten or more, 15 or more, 20 or more, 25 or more, 30 or more, etc. (through to and including all) of the contiguous nucleotides or bp of the *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* nucleotide sequences depicted in Figure 9 (or nucleotides complementary thereto), linked to the 5' end of a target-specific (e.g., a gene-specific) primer molecule. As noted above, such primer nucleic acid molecules may optionally further comprise one, two, three, four, five, or more additional nucleotide bases at their 5' ends, and preferably will comprise four guanines at their 5' ends. Other primer molecules comprising the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* sequences depicted in Figure 9, or portions thereof, may be made by one of ordinary skill without resorting to undue experimentation in accordance with the guidance provided herein.

The primers of the invention described herein are useful in producing PCR fragments having a nucleic acid molecule of interest flanked at each end by a recombination site sequence (as described in detail below in Example 9), for use in cloning of PCR-amplified DNA fragments using the recombination system of the invention (as described in detail below in Examples 8, 19 and 21-25).

### Vectors

The invention also relates to vectors comprising one or more of the nucleic acid molecules of the invention, as described herein. In accordance with the invention, any vector may be used to construct the vectors of the invention. In

particular, vectors known in the art and those commercially available (and variants or derivatives thereof) may in accordance with the invention be engineered to include one or more nucleic acid molecules encoding one or more recombination sites (or portions thereof), or mutants, fragments, or derivatives thereof, for use  
5 in the methods of the invention. Such vectors may be obtained from, for example, Vector Laboratories Inc., InVitrogen, Promega, Novagen, New England Biolabs, Clontech, Roche, Pharmacia, EpiCenter, OriGenes Technologies Inc., Stratagene, Perkin Elmer, Pharmingen, Life Technologies, Inc., and Research Genetics. Such vectors may then for example be used for cloning or subcloning nucleic acid  
10 molecules of interest. General classes of vectors of particular interest include prokaryotic and/or eukaryotic cloning vectors, Expression Vectors, fusion vectors, two-hybrid or reverse two-hybrid vectors, shuttle vectors for use in different hosts, mutagenesis vectors, transcription vectors, vectors for receiving large inserts and the like.

15 Other vectors of interest include viral origin vectors (M13 vectors, bacterial phage  $\lambda$  vectors, bacteriophage P1 vectors, adenovirus vectors, herpesvirus vectors, retrovirus vectors, phage display vectors, combinatorial library vectors), high, low, and adjustable copy number vectors, vectors which have compatible replicons for use in combination in a single host (pACYC184 and pBR322) and  
20 eukaryotic episomal replication vectors (pCDM8).

Particular vectors of interest include prokaryotic Expression Vectors such as pcDNA II, pSL301, pSE280, pSE380, pSE420, pTrcHisA, B, and C, pRSET A, B, and C (Invitrogen, Inc.), pGEMEX-1, and pGEMEX-2 (Promega, Inc.), the pET vectors (Novagen, Inc.), pTrc99A, pKK223-3, the pGEX vectors, pEZZ18,  
25 pRIT2T, and pMC1871 (Pharmacia, Inc.), pKK233-2 and pKK388-1 (Clontech, Inc.), and pProEx-HT (Life Technologies, Inc.) and variants and derivatives thereof. Destination Vectors can also be made from eukaryotic Expression Vectors such as pFastBac, pFastBac HT, pFastBac DUAL, pSFV, and pTet-Splice (Life Technologies, Inc.), pEUK-C1, pPUR, pMAM, pMAMneo, pBI101,  
30 pBI121, pDR2, pCMVEBNA, and pYACneo (Clontech), pSVK3, pSVL, pMSG, pCH110, and pKK232-8 (Pharmacia, Inc.), p3'SS, pXT1, pSG5, pPbac, pMbac, pMC1neo, and pOG44 (Stratagene, Inc.), and pYES2, pAC360, pBlueBacHis A,

B, and C, pVL1392, pBsueBacIII, pCDM8, pcDNA1, pZeoSV, pcDNA3 pREP4, pCEP4, and pEBVHis (Invitrogen, Inc.) and variants or derivatives thereof.

Other vectors of particular interest include pUC18, pUC19, pBlueScript, pSPORT, cosmid, phagemids, YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes), MACs (mammalian artificial chromosomes), pQE70, pQE60, pQE9 (Quiagen), pBS vectors, PhageScript vectors, BlueScript vectors, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene), pcDNA3 (Invitrogen), pGEX, pTrsfus, pTrc99A, pET-5, pET-9, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia), pSPORT1, pSPORT2, pCMVSPORT2.0 and pSVSPORT1 (Life Technologies, Inc.) and variants or derivatives thereof.

Additional vectors of interest include pTrxFus, pThioHis, pLEX, pTrcHis, pTrcHis2, pRSET, pBlueBacHis2, pcDNA3.1/His, pcDNA3.1(-)/Myc-His, pSecTag, pEBVHis, pPIC9K, pPIC3.5K, pAO815, pPICZ, pPICZ $\alpha$ , pGAPZ, pGAPZ $\alpha$ , pBlueBac4.5, pBlueBacHis2, pMelBac, pSinRep5, pSinHis, pIND, pIND(SP1), pVgRXR, pcDNA2.1, pYES2, pZerO1.1, pZerO-2.1, pCR-Blunt, pSE280, pSE380, pSE420, pVL1392, pVL1393, pCDM8, pcDNA1.1, pcDNA1.1/Amp, pcDNA3.1, pcDNA3.1/Zeo, pSe,SV2, pRc/CMV2, pRc/RSV, pREP4, pREP7, pREP8, pREP9, pREP10, pCEP4, pEBVHis, pCR3.1, pCR2.1, pCR3.1-Uni, and pCRBac from Invitrogen;  $\lambda$ ExCell,  $\lambda$ gt11, pTrc99A, pKK223-3, pGEX-1 $\lambda$ T, pGEX-2T, pGEX-2TK, pGEX-4T-1, pGEX-4T-2, pGEX-4T-3, pGEX-3X, pGEX-5X-1, pGEX-5X-2, pGEX-5X-3, pEZZ18, pRIT2T, pMC1871, pSVK3, pSVL, pMSG, pCH110, pKK232-8, pSL1180, pNEO, and pUC4K from Pharmacia; pSCREEN-1b(+), pT7Blue(R), pT7Blue-2, pCITE-4abc(+), pOCUS-2, pTag, pET-32 LIC, pET-30 LIC, pBAC-2cp LIC, pBACgus-2cp LIC, pT7Blue-2 LIC, pT7Blue-2,  $\lambda$ SCREEN-1,  $\lambda$ BlueSTAR, pET-3abcd, pET-7abc, pET9abcd, pET11abcd, pET12abc, pET-14b, pET-15b, pET-16b, pET-17b-pET-17xb, pET-19b, pET-20b(+), pET-21abcd(+), pET-22b(+), pET-23abcd(+), pET-24abcd(+), pET-25b(+), pET-26b(+), pET-27b(+), pET-28abc(+), pET-29abc(+), pET-30abc(+), pET-31b(+), pET-32abc(+), pET-33b(+), pBAC-1, pBACgus-1, pBAC4x-1, pBACgus4x-1, pBAC-3cp, pBACgus-2cp, pBACsurf-1, plg, Signal plg, pYX, Selecta Vecta-Neo, Selecta Vecta - Hyg, and Selecta Vecta - Gpt from Novagen; pLexA, pB42AD, pGBT9, pAS2-1,

pGAD424, pACT2, pGAD GL, pGAD GH, pGAD10, pGilda, pEZM3, pEGFP, pEGFP-1, pEGFP-N, pEGFP-C, pEBFP, pGFPuv, pGFP, p6xHis-GFP, pSEAP2-Basic, pSEAP2-Contral, pSEAP2-Promoter, pSEAP2-Enhancer, p $\beta$ gal-Basic, p $\beta$ gal-Control, p $\beta$ gal-Promoter, p $\beta$ gal-Enhancer, pCMV $\beta$ , pTet-Off, pTet-On, pTK-Hyg, pRetro-Off, pRetro-On, pIRES1neo, pIRES1hyg, pLXSN, pLNCX, pLAPSN, pMAMneo, pMAMneo-CAT, pMAMneo-LUC, pPUR, pSV2neo, pYEX4T-1/2/3, pYEX-S1, pBacPAK-His, pBacPAK8/9, pAcUW31, BacPAK6, pTriplEx,  $\lambda$ gt10,  $\lambda$ gt11, pWE15, and  $\lambda$ TriplEx from Clontech; Lambda ZAP II, pBK-CMV, pBK-RSV, pBluescript II KS +/-, pBluescript II SK +/-, pAD-GAL4, pBD-GAL4 Cam, pSurfscrip, Lambda FIX II, Lambda DASH, Lambda EMBL3, Lambda EMBL4, SuperCos, pCR-Script Amp, pCR-Script Cam, pCR-Script Direct, pBS +/-, pBC KS +/-, pBC SK +/-, Phagescript, pCAL-n-EK, pCAL-n, pCAL-c, pCAL-kc, pET-3abcd, pET-11abcd, pSPUTK, pESP-1, pCMVLacI, pOPRSVI/MCS, pOPI3 CAT, pXT1, pSG5, pPbac, pMbac, pMC1neo, pMC1neo Poly A, pOG44, pOG45, pFRT $\beta$ GAL, pNEO $\beta$ GAL, pRS403, pRS404, pRS405, pRS406, pRS413, pRS414, pRS415, and pRS416 from Stratagene.

Two-hybrid and reverse two-hybrid vectors of particular interest include pPC86, pDBLeu, pDBTrp, pPC97, p2.5, pGAD1-3, pGAD10, pACt, pACT2, pGADGL, pGADGH, pAS2-1, pGAD424, pGBT8, pGBT9, pGAD-GAL4, pLexA, pBD-GAL4, pHISi, pHISi-1, placZi, pB42AD, pDG202, pJK202, pJG4-5, pNLexA, pYESTrp and variants or derivatives thereof.

Yeast Expression Vectors of particular interest include pESP-1, pESP-2, pESC-His, pESC-Trp, pESC-URA, pESC-Leu (Stratagene), pRS401, pRS402, pRS411, pRS412, pRS421, pRS422, and variants or derivatives thereof.

According to the invention, the vectors comprising one or more nucleic acid molecules encoding one or more recombination sites, or mutants, variants, fragments, or derivatives thereof, may be produced by one of ordinary skill in the art without resorting to undue experimentation using standard molecular biology methods. For example, the vectors of the invention may be produced by introducing one or more of the nucleic acid molecules encoding one or more recombination sites (or mutants, fragments, variants or derivatives thereof) into one or more of the vectors described herein, according to the methods described,

for example, in Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982). In a related aspect of the invention, the vectors may be engineered to contain, in addition to one or more nucleic acid molecules encoding one or more recombination sites (or portions thereof), one or more additional physical or functional nucleotide sequences, such as those encoding one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (e.g., one or more promoters, enhancers, or repressors), one or more selection markers or modules, one or more genes or portions of genes encoding a protein or polypeptide of interest, one or more translational signal sequences, one or more nucleotide sequences encoding a fusion partner protein or peptide (e.g., GST, His, or thioredoxin), one or more origins of replication, and one or more 5' or 3' polynucleotide tails (particularly a poly-G tail). According to this aspect of the invention, the one or more recombination site nucleotide sequences (or portions thereof) may optionally be operably linked to the one or more additional physical or functional nucleotide sequences described herein.

Preferred vectors according to this aspect of the invention include, but are not limited to: pENTR1A (Figures 10A and 10B), pENTR2B (Figures 11A and 11B), pENTR3C (Figures 12A and 12B), pENTR4 (Figures 13A and 13B), pENTR5 (Figures 14A and 14B), pENTR6 (Figures 15A and 15B), pENTR7 (Figures 16A and 16B), pENTR8 (Figures 17A and 17B), pENTR9 (Figures 18A and 18B), pENTR10 (Figures 19A and 19B), pENTR11 (Figures 20A and 20B), pDEST1 (Figures 21A-D), pDEST2 (Figure 22A-D), pDEST3 (Figure 23A-D), pDEST4 (Figure 24A-D), pDEST5 (Figure 25A-D), pDEST6 (Figure 26A-D), pDEST7 (Figure 27A-C), pDEST8 (Figure 28A-D), pDEST9 (Figure 29A-E), pDEST10 (Figure 30A-D), pDEST11 (Figure 31A-D), pDEST12.2 (also known as pDEST12) (Figure 32A-D), pDEST13 (Figure 33A-C), pDEST14 (Figure 34A-D), pDEST15 (Figure 35A-D), pDEST16 (Figure 36A-D), pDEST17 (Figure 37A-D), pDEST18 (Figure 38A-D), pDEST19 (Figure 39A-D), pDEST20 (Figure 40A-D), pDEST21 (Figure 41A-E), pDEST22 (Figure 42A-D), pDEST23 (Figure 43A-D), pDEST24 (Figure 44A-D), pDEST25 (Figure 45A-D), pDEST26 (Figure 46A-D), pDEST27 (Figure 47A-D), pEXP501 (also known

as pCMVSPORT6) (Figure 48A-B), pDONR201 (also known as pENTR21 attP vector or pAttPkan Donor Vector) (Figure 49), pDONR202 (Figure 50), pDONR203 (also known as pEZ15812) (Figure 51), pDONR204 (Figure 52), pDONR205 (Figure 53), pDONR206 (also known as pENTR22 attP vector or pAttPgen Donor Vector) (Figure 54), pMAB58 (Figure 87), pMAB62 (Figure 88), pDEST28 (Figure 90), pDEST29 (Figure 91), pDEST30 (Figure 92), pDEST31 (Figure 93), pDEST32 (Figure 94), pDEST33 (Figure 95), pDEST34 (Figure 96), pDONR207 (Figure 97), pMAB85 (Figure 98), pMAB86 (Figure 99), and fragments, mutants, variants, and derivatives thereof. However, it will be understood by one of ordinary skill that the present invention also encompasses other vectors not specifically designated herein, which comprise one or more of the isolated nucleic acid molecules of the invention encoding one or more recombination sites or portions thereof (or mutants, fragments, variants or derivatives thereof), and which may further comprise one or more additional physical or functional nucleotide sequences described herein which may optionally be operably linked to the one or more nucleic acid molecules encoding one or more recombination sites or portions thereof. Such additional vectors may be produced by one of ordinary skill according to the guidance provided in the present specification.

### ***Polymerases***

Preferred polypeptides having reverse transcriptase activity (*i.e.*, those polypeptides able to catalyze the synthesis of a DNA molecule from an RNA template) for use in accordance with the present invention include, but are not limited to Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase, Rous Sarcoma Virus (RSV) reverse transcriptase, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Rous Associated Virus (RAV) reverse transcriptase, Myeloblastosis Associated Virus (MAV) reverse transcriptase, Human Immunodeficiency Virus (HIV) reverse transcriptase, retroviral reverse transcriptase, retrotransposon reverse transcriptase, hepatitis B reverse transcriptase, cauliflower mosaic virus reverse transcriptase and bacterial reverse transcriptase. Particularly preferred are those polypeptides having reverse



transcriptase activity that are also substantially reduced in RNase H activity (*i.e.*, “RNase H” polypeptides). By a polypeptide that is “substantially reduced in RNase H activity” is meant that the polypeptide has less than about 20%, more preferably less than about 15%, 10% or 5%, and most preferably less than about 2%, of the RNase H activity of a wildtype or RNase H<sup>-</sup> enzyme such as wildtype M-MLV reverse transcriptase. The RNase H activity may be determined by a variety of assays, such as those described, for example, in U.S. Patent No. 5,244,797, in Kotewicz, M.L. *et al.*, *Nucl. Acids Res.* 16:265 (1988) and in Gerard, G.F., *et al.*, *FOCUS* 14(5):91 (1992), the disclosures of all of which are fully incorporated herein by reference. Suitable RNase H<sup>-</sup> polypeptides for use in the present invention include, but are not limited to, M-MLV H<sup>-</sup> reverse transcriptase, RSV H<sup>-</sup> reverse transcriptase, AMV H<sup>-</sup> reverse transcriptase, RAV H<sup>-</sup> reverse transcriptase, MAV H<sup>-</sup> reverse transcriptase, HIV H<sup>-</sup> reverse transcriptase, THERMOSCRIPT™ reverse transcriptase and THERMOSCRIPT™ II reverse transcriptase, and SUPERScript™ I reverse transcriptase and SUPERScript™ II reverse transcriptase, which are obtainable, for example, from Life Technologies, Inc. (Rockville, Maryland). See generally published PCT application WO 98/47912.

Other polypeptides having nucleic acid polymerase activity suitable for use in the present methods include thermophilic DNA polymerases such as DNA polymerase I, DNA polymerase III, Klenow fragment, T7 polymerase, and T5 polymerase, and thermostable DNA polymerases including, but not limited to, *Thermus thermophilus* (*Tth*) DNA polymerase, *Thermus aquaticus* (*Taq*) DNA polymerase, *Thermotoga neopolitana* (*Tne*) DNA polymerase, *Thermotoga maritima* (*Tma*) DNA polymerase, *Thermococcus litoralis* (*Tli* or VENT®) DNA polymerase, *Pyrococcus furiosus* (*Pfu*) DNA polymerase, *Pyrococcus* species GB-D (or DEEPVENT®) DNA polymerase, *Pyrococcus woosii* (*Pwo*) DNA polymerase, *Bacillus stearothermophilus* (*Bst*) DNA polymerase, *Sulfolobus acidocaldarius* (*Sac*) DNA polymerase, *Thermoplasma acidophilum* (*Tac*) DNA polymerase, *Thermus flavus* (*Tfi/Tub*) DNA polymerase, *Thermus ruber* (*Tru*) DNA polymerase, *Thermus brockianus* (DYNAZYME®) DNA polymerase, *Methanobacterium thermoautotrophicum* (*Mth*) DNA polymerase, and mutants,

variants and derivatives thereof. Such polypeptides are available commercially, for example from Life Technologies, Inc. (Rockville, MD), New England BioLabs (Beverly, MA), and Sigma/Aldrich (St. Louis, MO).

### Host Cells

The invention also relates to host cells comprising one or more of the nucleic acid molecules or vectors of the invention, particularly those nucleic acid molecules and vectors described in detail herein. Representative host cells that may be used according to this aspect of the invention include, but are not limited to, bacterial cells, yeast cells, plant cells and animal cells. Preferred bacterial host cells include *Escherichia* spp. cells (particularly *E. coli* cells and most particularly *E. coli* strains DH10B, Stbl2, DH5 $\alpha$ , DB3, DB3.1 (preferably *E. coli* LIBRARY EFFICIENCY $\otimes$  DB3.1<sup>TM</sup> Competent Cells; Life Technologies, Inc., Rockville, MD), DB4 and DB5; see U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, the disclosure of which is incorporated by reference herein in its entirety), *Bacillus* spp. cells (particularly *B. subtilis* and *B. megaterium* cells), *Streptomyces* spp. cells, *Erwinia* spp. cells, *Klebsiella* spp. cells, *Serratia* spp. cells (particularly *S. marcessans* cells), *Pseudomonas* spp. cells (particularly *P. aeruginosa* cells), and *Salmonella* spp. cells (particularly *S. typhimurium* and *S. typhi* cells). Preferred animal host cells include insect cells (most particularly *Drosophila melanogaster* cells, *Spodoptera frugiperda* Sf9 and Sf21 cells and *Trichoplusia* High-Five cells), nematode cells (particularly *C. elegans* cells), avian cells, amphibian cells (particularly *Xenopus laevis* cells), reptilian cells, and mammalian cells (most particularly CHO, COS, VERO, BHK and human cells). Preferred yeast host cells include *Saccharomyces cerevisiae* cells and *Pichia pastoris* cells. These and other suitable host cells are available commercially, for example from Life Technologies, Inc. (Rockville, Maryland), American Type Culture Collection (Manassas, Virginia), and Agricultural Research Culture Collection (NRRL; Peoria, Illinois).

Methods for introducing the nucleic acid molecules and/or vectors of the invention into the host cells described herein, to produce host cells comprising one or more of the nucleic acid molecules and/or vectors of the invention, will be

familiar to those of ordinary skill in the art. For instance, the nucleic acid molecules and/or vectors of the invention may be introduced into host cells using well known techniques of infection, transduction, transfection, and transformation. The nucleic acid molecules and/or vectors of the invention may be introduced alone or in conjunction with other the nucleic acid molecules and/or vectors. Alternatively, the nucleic acid molecules and/or vectors of the invention may be introduced into host cells as a precipitate, such as a calcium phosphate precipitate, or in a complex with a lipid. Electroporation also may be used to introduce the nucleic acid molecules and/or vectors of the invention into a host. Likewise, such molecules may be introduced into chemically competent cells such as *E. coli*. If the vector is a virus, it may be packaged *in vitro* or introduced into a packaging cell and the packaged virus may be transduced into cells. Hence, a wide variety of techniques suitable for introducing the nucleic acid molecules and/or vectors of the invention into cells in accordance with this aspect of the invention are well known and routine to those of skill in the art. Such techniques are reviewed at length, for example, in Sambrook, J., *et al.*, *Molecular Cloning, a Laboratory Manual, 2nd Ed.*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp. 16.30-16.55 (1989), Watson, J.D., *et al.*, *Recombinant DNA, 2nd Ed.*, New York: W.H. Freeman and Co., pp. 213-234 (1992), and Winnacker, E.-L., *From Genes to Clones*, New York: VCH Publishers (1987), which are illustrative of the many laboratory manuals that detail these techniques and which are incorporated by reference herein in their entireties for their relevant disclosures.

### ***Polypeptides***

In another aspect, the invention relates to polypeptides encoded by the nucleic acid molecules of the invention (including polypeptides and amino acid sequences encoded by all possible reading frames of the nucleic acid molecules of the invention), and to methods of producing such polypeptides. Polypeptides of the present invention include purified or isolated natural products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, insect, mammalian, avian and higher plant cells.

The polypeptides of the invention may be produced by synthetic organic chemistry, and are preferably produced by standard recombinant methods, employing one or more of the host cells of the invention comprising the vectors or isolated nucleic acid molecules of the invention. According to the invention, polypeptides are produced by cultivating the host cells of the invention (which comprise one or more of the nucleic acid molecules of the invention, preferably contained within an Expression Vector) under conditions favoring the expression of the nucleotide sequence contained on the nucleic acid molecule of the invention, such that the polypeptide encoded by the nucleic acid molecule of the invention is produced by the host cell. As used herein, "conditions favoring the expression of the nucleotide sequence" or "conditions favoring the production of a polypeptide" include optimal physical (e.g., temperature, humidity, etc.) and nutritional (e.g., culture medium, ionic) conditions required for production of a recombinant polypeptide by a given host cell. Such optimal conditions for a variety of host cells, including prokaryotic (bacterial), mammalian, insect, yeast, and plant cells will be familiar to one of ordinary skill in the art, and may be found, for example, in Sambrook, J., *et al.*, *Molecular Cloning, A Laboratory Manual, 2nd Ed.*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, (1989), Watson, J.D., *et al.*, *Recombinant DNA, 2nd Ed.*, New York: W.H. Freeman and Co., and Winnacker, E.-L., *From Genes to Clones*, New York: VCH Publishers (1987).

In some aspects, it may be desirable to isolate or purify the polypeptides of the invention (e.g., for production of antibodies as described below), resulting in the production of the polypeptides of the invention in isolated form. The polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods of protein purification that are routine in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. For example, His6 or GST fusion tags on polypeptides made by the methods of the invention may be isolated using appropriate affinity chromatography matrices which bind polypeptides bearing

His6 or GST tags, as will be familiar to one of ordinary skill in the art. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Isolated polypeptides of the invention include those comprising the amino acid sequences encoded by one or more of the reading frames of the polynucleotides comprising one or more of the recombination site-encoding nucleic acid molecules of the invention, including those encoding *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* having the nucleotide sequences set forth in Figure 9 (or nucleotide sequences complementary thereto), or fragments, variants, mutants and derivatives thereof; the complete amino acid sequences encoded by the polynucleotides contained in the deposited clones described herein; the amino acid sequences encoded by polynucleotides which hybridize under stringent hybridization conditions to polynucleotides having the nucleotide sequences encoding the recombination site sequences of the invention as set forth in Figure 9 (or a nucleotide sequence complementary thereto); or a peptide or polypeptide comprising a portion or a fragment of the above polypeptides. The invention also relates to additional polypeptides having one or more additional amino acids linked (typically by peptidyl bonds to form a nascent polypeptide) to the polypeptides encoded by the recombination site nucleotide sequences or the deposited clones. Such additional amino acid residues may comprise one or more functional peptide sequences, for example one or more fusion partner peptides (e.g., GST, His<sub>6</sub>, Trx, etc.) and the like.

As used herein, the terms "protein," "peptide," "oligopeptide" and "polypeptide" are considered synonymous (as is commonly recognized) and each term can be used interchangeably as the context requires to indicate a chain of two or more amino acids, preferably five or more amino acids, or more preferably ten

or more amino acids, coupled by (a) peptidyl linkage(s), unless otherwise defined in the specific contexts below. As is commonly recognized in the art, all polypeptide formulas or sequences herein are written from left to right and in the direction from amino terminus to carboxy terminus.

5 It will be recognized by those of ordinary skill in the art that some amino acid sequences of the polypeptides of the invention can be varied without significant effect on the structure or function of the polypeptides. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine structure and activity. In general, it is possible to replace residues which form the tertiary structure, provided that  
10 residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the polypeptide.

Thus, the invention further includes variants of the polypeptides of the invention, including allelic variants, which show substantial structural homology  
15 to the polypeptides described herein, or which include specific regions of these polypeptides such as the portions discussed below. Such mutants may include deletions, insertions, inversions, repeats, and type substitutions (for example, substituting one hydrophilic residue for another, but not strongly hydrophilic for  
20 strongly hydrophobic as a rule). Small changes or such "neutral" or "conservative" amino acid substitutions will generally have little effect on activity.

Typical conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxylated residues Ser and Thr; exchange of the acidic residues Asp and Glu;  
25 substitution between the amidated residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe and Tyr.

Thus, the fragment, derivative or analog of the polypeptides of the invention, such as those comprising peptides encoded by the recombination site nucleotide sequences described herein, may be (i) one in which one or more of the amino acid  
30 residues are substituted with a conservative or non-conservative amino acid residue (preferably a conservative amino acid residue), and such substituted amino acid residue may be encoded by the genetic code or may be an amino acid (*e.g.*,

desmosine, citrulline, ornithine, etc.) that is not encoded by the genetic code; (ii) one in which one or more of the amino acid residues includes a substituent group (e.g., a phosphate, hydroxyl, sulfate or other group) in addition to the normal "R" group of the amino acid; (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which additional amino acids are fused to the mature polypeptide, such as an immunoglobulin Fc region peptide, a leader or secretory sequence, a sequence which is employed for purification of the mature polypeptide (such as GST) or a proprotein sequence. Such fragments, derivatives and analogs are intended to be encompassed by the present invention, and are within the scope of those skilled in the art from the teachings herein and the state of the art at the time of invention.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. Recombinantly produced versions of the polypeptides of the invention can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). As used herein, the term "substantially purified" means a preparation of an individual polypeptide of the invention wherein at least 50%, preferably at least 60%, 70%, or 75% and more preferably at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% (by mass) of contaminating proteins (*i.e.*, those that are not the individual polypeptides described herein or fragments, variants, mutants or derivatives thereof) have been removed from the preparation.

The polypeptides of the present invention include those which are at least about 50% identical, at least 60% identical, at least 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical, to the polypeptides described herein. For example, preferred *attB1*-containing polypeptides of the invention include those that are at least about 50% identical, at least 60% identical, at least 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical,

to the polypeptide(s) encoded by the three reading frames of a polynucleotide comprising a nucleotide sequence of *attB1* having a nucleic acid sequence as set forth in Figure 9 (or a nucleic acid sequence complementary thereto), to a polypeptide encoded by a polynucleotide contained in the deposited cDNA clones described herein, or to a polypeptide encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence of *attB1* having a nucleic acid sequence as set forth in Figure 9 (or a nucleic acid sequence complementary thereto). Analogous polypeptides may be prepared that are at least about 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical, to the *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* polypeptides of the invention as depicted in Figure 9. The present polypeptides also include portions or fragments of the above-described polypeptides with at least 5, 10, 15, 20, or 25 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 65% "identical" to a reference amino acid sequence of a given polypeptide of the invention is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to 35 amino acid alterations per each 100 amino acids of the reference amino acid sequence of a given polypeptide of the invention. In other words, to obtain a polypeptide having an amino acid sequence at least 65% identical to a reference amino acid sequence, up to 35% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 35% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino (N-) or carboxy (C-) terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether a given amino acid sequence is, for example, at least 65% identical to the amino acid sequence of a given polypeptide of the invention can be determined



conventionally using known computer programs such as those described above for nucleic acid sequence identity determinations, or more preferably using the CLUSTAL W program (Thompson, J.D., *et al.*, *Nucleic Acids Res.* 22:4673-4680 (1994)).

5       The polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. In addition, as described in detail below, the polypeptides of the present invention can be used to raise polyclonal and monoclonal antibodies which are useful in a variety of assays for detecting  
10       protein expression, localization, detection of interactions with other molecules, or for the isolation of a polypeptide (including a fusion polypeptide) of the invention.

      In another aspect, the present invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention, which may be used to raise antibodies, particularly monoclonal antibodies, that bind  
15       specifically to a one or more of the polypeptides of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. These immunogenic epitopes are believed to be confined to a few loci on the molecule.  
20       On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes (*see, e.g.*, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1983)).

      As to the selection of peptides or polypeptides bearing an antigenic epitope  
25       (*i.e.*, that contain a region of a protein molecule to which an antibody can bind), it is well-known in the art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein (*see, e.g.*, Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983)). Peptides capable of eliciting protein-reactive sera are  
30       frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are not confined to the immunodominant regions of intact proteins (*i.e.*, immunogenic epitopes) or to the amino or carboxy

termini. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer peptides, especially those containing proline residues, usually are effective (Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983)).

5        Epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least five, more preferably at least seven or more amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of a polypeptide of the invention, containing about 30 to about 50 amino acids, or any length up to and including the entire amino acid sequence of a given polypeptide of the invention, also are considered epitope-bearing peptides or polypeptides of the invention and also are useful for inducing antibodies that react with the mimicked protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues and highly hydrophobic sequences are preferably avoided); sequences containing proline residues are particularly preferred.

15        Non-limiting examples of epitope-bearing polypeptides or peptides that can be used to generate antibodies specific for the polypeptides of the invention include certain epitope-bearing regions of the polypeptides comprising amino acid sequences encoded by polynucleotides comprising one or more of the recombination site-encoding nucleic acid molecules of the invention, including those encoding *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* having the nucleotide sequences set forth in Figure 9 (or a nucleotide sequence complementary thereto); the complete amino acid sequences encoded by the three reading frames of the polynucleotides contained in the deposited clones described herein; and the amino acid sequences encoded by all reading frames of polynucleotides which hybridize under stringent hybridization conditions to polynucleotides having the nucleotide sequences encoding the recombination site sequences (or portions thereof) of the invention as set forth in Figure 9 (or a nucleic acid sequence complementary thereto). Other epitope-bearing polypeptides or peptides that may be used to generate antibodies specific for the polypeptides

of the invention will be apparent to one of ordinary skill in the art based on the primary amino acid sequences of the polypeptides of the invention described herein, via the construction of Kyte-Doolittle hydrophilicity and Jameson-Wolf antigenic index plots of the polypeptides of the invention using, for example, 5 PROTEAN computer software (DNASTAR, Inc.; Madison, Wisconsin).

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means for making peptides or polypeptides including recombinant means using nucleic acid molecules of the invention. For instance, a short epitope-bearing amino acid sequence may be fused to a larger 10 polypeptide which acts as a carrier during recombinant production and purification, as well as during immunization to produce anti-peptide antibodies. Epitope-bearing peptides also may be synthesized using known methods of chemical synthesis (*see, e.g.*, U.S. Patent No. 4,631,211 and Houghten, R. A., *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985), both of which are incorporated 15 by reference herein in their entireties).

As one of skill in the art will appreciate, the polypeptides of the present invention and epitope-bearing fragments thereof may be immobilized onto a solid support, by techniques that are well-known and routine in the art. By "solid support" is intended any solid support to which a peptide can be immobilized. 20 Such solid supports include, but are not limited to nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtitre plates. Linkage of the peptide of the invention to a solid support can be accomplished by attaching one or both ends of the peptide to the support. Attachment may also be made at one or more 25 internal sites in the peptide. Multiple attachments (both internal and at the ends of the peptide) may also be used according to the invention. Attachment can be via an amino acid linkage group such as a primary amino group, a carboxyl group, or a sulfhydryl (SH) group or by chemical linkage groups such as with cyanogen bromide (CNBr) linkage through a spacer. For non-covalent attachments to the support, addition of an affinity tag sequence to the peptide can be used such as 30 GST (Smith, D.B., and Johnson, K.S., *Gene* 67:31 (1988)), polyhistidines (Hochuli, E., *et al.*, *J. Chromatog.* 411:77 (1987)), or biotin. Such affinity tags

may be used for the reversible attachment of the peptide to the support. Such immobilized polypeptides or fragments may be useful, for example, in isolating antibodies directed against one or more of the polypeptides of the invention, or other proteins or peptides that recognize other proteins or peptides that bind to one or more of the polypeptides of the invention, as described below.

As one of skill in the art will also appreciate, the polypeptides of the present invention and the epitope-bearing fragments thereof described herein can be combined with one or more fusion partner proteins or peptides, or portions thereof, including but not limited to GST, His<sub>6</sub>, Trx, and portions of the constant domain of immunoglobulins (Ig), resulting in chimeric or fusion polypeptides. These fusion polypeptides facilitate purification of the polypeptides of the invention (EP 0 394 827; Traunecker *et al.*, *Nature* 331:84-86 (1988)) for use in analytical or diagnostic (including high-throughput) format.

### ***Antibodies***

In another aspect, the invention relates to antibodies that recognize and bind to the polypeptides (or epitope-bearing fragments thereof) or nucleic acid molecules (or portions thereof) of the invention. In a related aspect, the invention relates to antibodies that recognize and bind to one or more polypeptides encoded by all reading frames of one or more recombination site nucleic acid sequences or portions thereof, or to one or more nucleic acid molecules comprising one or more recombination site nucleic acid sequences or portions thereof, including but not limited to *att* sites (including *attB*1, *attB*2, *attP*1, *attP*2, *attL*1, *attL*2, *attR*1, *attR*2 and the like), *lox* sites (*e.g.*, *loxP*, *loxP*511, and the like), FRT, and the like, or mutants, fragments, variants and derivatives thereof. See generally U.S. Patent No. 5,888,732, which is incorporated herein by reference in its entirety. The antibodies of the present invention may be polyclonal or monoclonal, and may be prepared by any of a variety of methods and in a variety of species according to methods that are well-known in the art. See, for instance, U.S. Patent No. 5,587,287; Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983); Wilson *et al.*, *Cell* 37: 767 (1984); and Bittle, F.J., *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985). Antibodies specific for any of the polypeptides or nucleic acid molecules described

herein, such as antibodies specifically binding to one or more of the polypeptides encoded by the recombination site nucleotide sequences, or one or more nucleic acid molecules, described herein or contained in the deposited clones, antibodies against fusion polypeptides (e.g., binding to fusion polypeptides between one or more of the fusion partner proteins and one or more of the recombination site polypeptides of the invention, as described herein), and the like, can be raised against the intact polypeptides or polynucleotides of the invention or one or more antigenic polypeptide fragments thereof.

As used herein, the term "antibody" (Ab) may be used interchangeably with the terms "polyclonal antibody" or "monoclonal antibody" (mAb), except in specific contexts as described below. These terms, as used herein, are meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')<sub>2</sub> fragments) which are capable of specifically binding to a polypeptide or nucleic acid molecule of the invention or a portion thereof. It will therefore be appreciated that, in addition to the intact antibodies of the invention, Fab, F(ab')<sub>2</sub> and other fragments of the antibodies described herein, and other peptides and peptide fragments that bind one or more polypeptides or polynucleotides of the invention, are also encompassed within the scope of the invention. Such antibody fragments are typically produced by proteolytic cleavage of intact antibodies, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Antibody fragments, and peptides or peptide fragments, may also be produced through the application of recombinant DNA technology or through synthetic chemistry.

Epitope-bearing peptides and polypeptides, and nucleic acid molecules or portions thereof, of the invention may be used to induce antibodies according to methods well known in the art, as generally described herein (*see, e.g., Sutcliffe, et al., supra; Wilson, et al., supra; and Bittle, F. J., et al., J. Gen. Virol. 66:2347-2354 (1985)*).

Polyclonal antibodies according to this aspect of the invention may be made by immunizing an animal with one or more of the polypeptides or nucleic acid molecules of the invention described herein or portions thereof according to standard techniques (*see, e.g., Harlow, E., and Lane, D., Antibodies: A*

*Laboratory Manual*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (1988); Kaufman, P.B., *et al.*, In: *Handbook of Molecular and Cellular Methods in Biology and Medicine*, Boca Raton, Florida: CRC Press, pp. 468-469 (1995)). For producing antibodies that recognize and bind to the polypeptides or nucleic acid molecules of the invention or portions thereof, animals may be immunized with free peptide or free nucleic acid molecules; however, antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as albumin, KLH, or tetanus toxoid (particularly for producing antibodies against the nucleic acid molecules of the invention or portions thereof; *see* Harlow and Lane, *supra*, at page 154), or to a solid phase carrier such as a latex or glass microbead. For instance, peptides containing cysteine may be coupled to carrier using a linker such as m-maleimidobenzoyl-N- hydroxysuccinimide ester (MBS), while other peptides may be coupled to carrier using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice may be immunized with either free (if the polypeptide immunogen is larger than about 25 amino acids in length) or carrier-coupled peptides or nucleic acid molecules, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg peptide, polynucleotide, or carrier protein, and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of antibody which can be detected, for example, by ELISA assay using free peptide or nucleic acid molecule adsorbed to a solid surface. In another approach, cells expressing one or more of the polypeptides or polynucleotides of the invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies, according to routine immunological methods. In yet another method, a preparation of one or more of the polypeptides or polynucleotides of the invention is prepared and purified as described herein, to render it substantially free of natural contaminants. Such a preparation may then be introduced into an animal in order to produce polyclonal antisera of greater specific activity. The titer of antibodies in serum from an immunized animal, regardless of the method of immunization used, may be increased by selection of anti-peptide or anti-polynucleotide antibodies, for

instance, by adsorption to the peptide or polynucleotide on a solid support and elution of the selected antibodies according to methods well known in the art.

In an alternative method, the antibodies of the present invention are monoclonal antibodies (or fragments thereof which bind to one or more of the polypeptides of the invention). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler *et al.*, *Nature* 256:495 (1975); Köhler *et al.*, *Eur. J. Immunol.* 6:511 (1976); Köhler *et al.*, *Eur. J. Immunol.* 6:292 (1976); Hammerling *et al.*, In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with a polypeptide or polynucleotide of the invention (or a fragment thereof), or with a cell expressing a polypeptide or polynucleotide of the invention (or a fragment thereof). The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP<sub>2</sub>O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al.* (*Gastroenterol.* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding one or more of the polypeptides or nucleic acid molecules of the invention, or fragments thereof. Hence, the present invention also provides hybridoma cells and cell lines producing monoclonal antibodies of the invention, particularly that recognize and bind to one or more of the polypeptides or nucleic acid molecules of the invention.

Alternatively, additional antibodies capable of binding to one or more of the polypeptides of the invention, or fragments thereof, may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, antibodies specific for one or more of the polypeptides or polynucleotides of the invention, prepared as described above, are used to immunize an animal, preferably a mouse. The splenocytes of such an

animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to an antibody specific for one or more of the polypeptides or polynucleotides of the invention can be blocked by polypeptides of the invention themselves. Such antibodies comprise anti-idiotypic antibodies to the antibodies recognizing one or more of the polypeptides or polynucleotides of the invention, and can be used to immunize an animal to induce formation of further antibodies specific for one or more of the polypeptides or polynucleotides of the invention.

For use, the antibodies of the invention may optionally be detectably labeled by covalent or non-covalent attachment of one or more labels, including but not limited to chromogenic, enzymatic, radioisotopic, isotopic, fluorescent, toxic, chemiluminescent, or nuclear magnetic resonance contrast agents or other labels.

Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

Examples of suitable radioisotopic labels include  $^3\text{H}$ ,  $^{111}\text{In}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ ,  $^{51}\text{Cr}$ ,  $^{57}\text{Co}$ ,  $^{58}\text{Co}$ ,  $^{59}\text{Fe}$ ,  $^{75}\text{Se}$ ,  $^{152}\text{Eu}$ ,  $^{90}\text{Y}$ ,  $^{67}\text{Cu}$ ,  $^{217}\text{Bi}$ ,  $^{211}\text{At}$ ,  $^{212}\text{Pb}$ ,  $^{47}\text{Sc}$ ,  $^{109}\text{Pd}$ , etc.  $^{111}\text{In}$  is a preferred isotope where in vivo imaging is used since it avoids the problem of dehalogenation of the  $^{125}\text{I}$  or  $^{131}\text{I}$ -labeled monoclonal antibody by the liver. In addition, this radionuclide has a more favorable gamma emission energy for imaging (Perkins *et al.*, *Eur. J. Nucl. Med.* 10:296-301 (1985); Carasquillo *et al.*, *J. Nucl. Med.* 28:281-287 (1987)). For example,  $^{111}\text{In}$  coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA has shown little uptake in non-tumorous tissues, particularly the liver, and therefore enhances specificity of tumor localization (Esteban *et al.*, *J. Nucl. Med.* 28:861-870 (1987)).

Examples of suitable non-radioactive isotopic labels include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Tr}$ , and  $^{56}\text{Fe}$ .

Examples of suitable fluorescent labels include an  $^{152}\text{Eu}$  label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a



phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, a green fluorescent protein (GFP) label, and a fluorescamine label.

Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin.

5        Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label.

10       Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

Typical techniques for binding the above-described labels to the antibodies of the invention are provided by Kennedy *et al.*, *Clin. Chim. Acta* 70:1-31 (1976), and Schurs *et al.*, *Clin. Chim. Acta* 81:1-40 (1977). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

15       It will be appreciated by one of ordinary skill that the antibodies of the present invention may alternatively be coupled to a solid support, to facilitate, for example, chromatographic and other immunological procedures using such solid phase-immobilized antibodies. Included among such procedures are the use of the antibodies of the invention to isolate or purify polypeptides comprising one or more epitopes encoded by the nucleic acid molecules of the invention (which may be fusion polypeptides or other polypeptides of the invention described herein), or to isolate or purify polynucleotides comprising one or more recombination site sequences of the invention or portions thereof. Methods for isolation and purification of polypeptides (and, by analogy, polynucleotides) by affinity chromatography, for example using the antibodies of the invention coupled to a solid phase support, are well-known in the art and will be familiar to one of ordinary skill. The antibodies of the invention may also be used in other applications, for example to cross-link or couple two or more proteins, polypeptides, polynucleotides, or portions thereof into a structural and/or functional complex. In one such use, an antibody of the invention may have two

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or more distinct epitope-binding regions that may bind, for example, a first polypeptide (which may be a polypeptide of the invention) at one epitope-binding region on the antibody and a second polypeptide (which may be a polypeptide of the invention) at a second epitope-binding region on the antibody, thereby bringing the first and second polypeptides into close proximity to each other such that the first and second polypeptides are able to interact structurally and/or functionally (as, for example, linking an enzyme and its substrate to carry out enzymatic catalysis, or linking an effector molecule and its receptor to carry out or induce a specific binding of the effector molecule to the receptor or a response to the effector molecule mediated by the receptor). Additional applications for the antibodies of the invention include, for example, the preparation of large-scale arrays of the antibodies, polypeptides, or nucleic acid molecules of the invention, or portions thereof, on a solid support, for example to facilitate high-throughput screening of protein or RNA expression by host cells containing nucleic acid molecules of the invention (known in the art as "chip array" protocols; *see, e.g.*, U.S. Patent Nos. 5,856,101, 5,837,832, 5,770,456, 5,744,305, 5,631,734, and 5,593,839, which are directed to production and use of chip arrays of polypeptides (including antibodies) and polynucleotides, and the disclosures of which are incorporated herein by reference in their entireties). By "solid support" is intended any solid support to which an antibody can be immobilized. Such solid supports include, but are not limited to nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polycarbonate, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtitre plates. Preferred are beads made of glass, latex or a magnetic material. Linkage of an antibody of the invention to a solid support can be accomplished by attaching one or both ends of the antibody to the support. Attachment may also be made at one or more internal sites in the antibody. Multiple attachments (both internal and at the ends of the antibody) may also be used according to the invention. Attachment can be via an amino acid linkage group such as a primary amino group, a carboxyl group, or a sulfhydryl (SH) group or by chemical linkage groups such as with cyanogen bromide (CNBr) linkage through a spacer. For non-covalent attachments, addition of an affinity tag sequence to the peptide can be used such as GST

(Smith, D.B., and Johnson, K.S., *Gene* 67:31 (1988)), polyhistidines (Hochuli, E., *et al.*, *J. Chromatog.* 411:77 (1987)), or biotin. Alternatively, attachment can be accomplished using a ligand which binds the Fc region of the antibodies of the invention, *e.g.*, protein A or protein G. Such affinity tags may be used for the reversible attachment of the antibodies to the support. Peptides may also be recognized via specific ligand-receptor interactions or using phage display methodologies that will be familiar to the skilled artisan, for their ability to bind polypeptides of the invention or fragments thereof.

### Kits

In another aspect, the invention provides kits which may be used in producing the nucleic acid molecules, polypeptides, vectors, host cells, and antibodies, and in the recombinational cloning methods, of the invention. Kits according to this aspect of the invention may comprise one or more containers, which may contain one or more of the nucleic acid molecules, primers, polypeptides, vectors, host cells, or antibodies of the invention. In particular, a kit of the invention may comprise one or more components (or combinations thereof) selected from the group consisting of one or more recombination proteins (*e.g.*, Int) or auxiliary factors (*e.g.* IHF and/or Xis) or combinations thereof, one or more compositions comprising one or more recombination proteins or auxiliary factors or combinations thereof (for example, GATEWAY™ LR Clonase™ Enzyme Mix or GATEWAY™ BP Clonase™ Enzyme Mix) one or more Destination Vector molecules (including those described herein), one or more Entry Clone or Entry Vector molecules (including those described herein), one or more primer nucleic acid molecules (particularly those described herein), one or more host cells (*e.g.* competent cells, such as *E. coli* cells, yeast cells, animal cells (including mammalian cells, insect cells, nematode cells, avian cells, fish cells, etc.), plant cells, and most particularly *E. coli* DB3, DB3.1 (preferably *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells; Life Technologies, Inc., Rockville, MD), DB4 and DB5; *see* U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, and the corresponding U.S. Utility Application No. \_\_\_\_\_ of Hartley *et al.*, entitled "Cells Resistant to Toxic Genes and Uses Thereof," filed

on even day herewith, the disclosures of which are incorporated by reference herein in its entirety), and the like. In related aspects, the kits of the invention may comprise one or more nucleic acid molecules encoding one or more recombination sites or portions thereof, such as one or more nucleic acid molecules comprising a nucleotide sequence encoding the one or more recombination sites (or portions thereof) of the invention, and particularly one or more of the nucleic acid molecules contained in the deposited clones described herein. Kits according to this aspect of the invention may also comprise one or more isolated nucleic acid molecules of the invention, one or more vectors of the invention, one or more primer nucleic acid molecules of the invention, and/or one or more antibodies of the invention. The kits of the invention may further comprise one or more additional containers containing one or more additional components useful in combination with the nucleic acid molecules, polypeptides, vectors, host cells, or antibodies of the invention, such as one or more buffers, one or more detergents, one or more polypeptides having nucleic acid polymerase activity, one or more polypeptides having reverse transcriptase activity, one or more transfection reagents, one or more nucleotides, and the like. Such kits may be used in any process advantageously using the nucleic acid molecules, primers, vectors, host cells, polypeptides, antibodies and other compositions of the invention, for example in methods of synthesizing nucleic acid molecules (*e.g.*, via amplification such as via PCR), in methods of cloning nucleic acid molecules (preferably via recombinational cloning as described herein), and the like.

#### ***Optimization of Recombinational Cloning System***

The usefulness of a particular nucleic acid molecule, or vector comprising a nucleic acid molecule, of the invention in methods of recombinational cloning may be determined by any one of a number of assay methods. For example, Entry and Destination vectors of the present invention may be assessed for their ability to function (*i.e.*, to mediate the transfer of a nucleic acid molecule, DNA segment, gene, cDNA molecule or library from a cloning vector to an Expression Vector) by carrying out a recombinational cloning reaction as described in more detail in the Examples below and as described in U.S. Application Nos. 08/663,002, filed

June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, 09/177,387, filed October 23, 1998, and 60/108,324, filed November 13, 1998, the disclosures of which are incorporated by reference herein in their entireties. Alternatively, the functionality of Entry and Destination Vectors prepared according to the invention may be assessed by examining the ability of these vectors to recombine and create cointegrate molecules, or to transfer a nucleic acid molecule of interest, using an assay such as that described in detail below in Example 19. Analogously, the formulation of compositions comprising one or more recombination proteins or combinations thereof, for example GATEWAY™ LR Clonase™ Enzyme Mix and GATEWAY™ BP Clonase™ Enzyme Mix, may be optimized using assays such as those described below in Example 18.

### *Uses*

There are a number of applications for the compositions, methods and kits of the present invention. These uses include, but are not limited to, changing vectors, targeting gene products to intracellular locations, cleaving fusion tags from desired proteins, operably linking nucleic acid molecules of interest to regulatory genetic sequences (*e.g.*, promoters, enhancers, and the like), constructing genes for fusion proteins, changing copy number, changing replicons, cloning into phages, and cloning, *e.g.*, PCR products, genomic DNAs, and cDNAs. In addition, the nucleic acid molecules, vectors, and host cells of the invention may be used in the production of polypeptides encoded by the nucleic acid molecules, in the production of antibodies directed against such polypeptides, in recombinational cloning of desired nucleic acid sequences, and in other applications that may be enhanced or facilitated by the use of the nucleic acid molecules, vectors, and host cells of the invention.

In particular, the nucleic acid molecules, vectors, host cells, polypeptides, antibodies, and kits of the invention may be used in methods of transferring one or more desired nucleic acid molecules or DNA segments, for example one or more genes, cDNA molecules or cDNA libraries, into a cloning or Expression Vector for use in transforming additional host cells for use in cloning or

amplification of, or expression of the polypeptide encoded by, the desired nucleic acid molecule or DNA segment. Such recombinational cloning methods which may advantageously use the nucleic acid molecules, vectors, and host cells of the invention, are described in detail in the Examples below, and in commonly owned  
5 U.S. Application Nos. 08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, 09/177,387, filed October 23, 1998, and 60/108,324, filed November 13, 1998, the disclosures of all of which are incorporated by reference herein in their entireties.

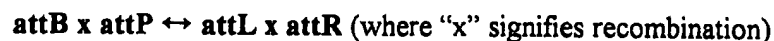
10 It will be understood by one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein are readily apparent from the description of the invention contained herein in view of information known to the ordinarily skilled artisan, and may be made  
15 without departing from the scope of the invention or any embodiment thereof. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

### Examples

#### ***Example 1: Recombination Reactions of Bacteriophage $\lambda$***

25 The *E. coli* bacteriophage  $\lambda$  can grow as a lytic phage, in which case the host cell is lysed, with the release of progeny virus. Alternatively, lambda can integrate into the genome of its host by a process called lysogenization (see Figure 60). In this lysogenic state, the phage genome can be transmitted to daughter cells for many generations, until conditions arise that trigger its excision from the genome.  
30 At this point, the virus enters the lytic part of its life cycle. The control of the switch between the lytic and lysogenic pathways is one of the best understood processes in molecular biology (M. Ptashne, *A Genetic Switch*, Cell Press, 1992).

The integrative and excisive recombination reactions of  $\lambda$ , performed *in vitro*, are the basis of Recombinational Cloning System of the present invention. They can be represented schematically as follows:



10                    The four att sites contain binding sites for the proteins that mediate the reactions. The wild type attP, attB, attL, and attR sites contain about 243, 25, 100, and 168 base pairs, respectively. The attB x attP reaction (hereinafter referred to as a "BP Reaction," or alternatively and equivalently as an "Entry Reaction" or a "Gateward Reaction") is mediated by the proteins Int and IHF. The attL x attR reaction (hereinafter referred to as an "LR Reaction," or alternatively and equivalently as a "Destination Reaction") is mediated by the proteins Int, IHF, and Xis. Int (integrase) and Xis (excisionase) are encoded by  
15                    the  $\lambda$  genome, while IHF (integration host factor) is an *E. coli* protein. For a general review of lambda recombination, see: A. Landy, *Ann. Rev. Biochem.* 58: 913-949 (1989).

20                    ***Example 2: Recombination Reactions of the Recombinational Cloning System***

                    The LR Reaction -- the exchange of a DNA segment from an Entry Clone to a Destination Vector -- is the *in vitro* version of the  $\lambda$  excision reaction:



30                    There is a practical imperative for this configuration: after an LR Reaction in one configuration of the present method, an att site usually separates a functional motif (such as a promoter or a fusion tag) from a nucleic acid molecule of interest in an Expression Clone, and the 25 bp attB site is much smaller than the attP, attL, and attR sites.

                    Note that the recombination reaction is conservative, i.e., there is no net synthesis or loss of base pairs. The DNA segments that flank the recombination

sites are merely switched. The wild type  $\lambda$  recombination sites are modified for purposes of the GATEWAY™ Cloning System, as follows:

To create certain preferred Destination Vectors, a part (43 bp) of attR was removed, to make the excisive reaction irreversible and more efficient (W. Bushman et al., *Science* 230: 906, 1985). The attR sites in preferred Destination Vectors of the invention are 125 bp in length. Mutations were made to the core regions of the att sites, for two reasons: (1) to eliminate stop codons, and (2) to ensure specificity of the recombination reactions (i.e., attR1 reacts only with attL1, attR2 reacts only with attL2, etc.).

Other mutations were introduced into the short (5 bp) regions flanking the 15 bp core regions of the attB sites to minimize secondary structure formation in single-stranded forms of attB plasmids, e.g., in phagemid ssDNA or in mRNA. Sequences of attB1 and attB2 to the left and right of a nucleic acid molecule of interest after it has been cloned into a Destination Vector are given in Figure 6.

Figure 61 illustrates how an Entry Clone and a Destination Vector recombine in the LR Reaction to form a co-integrate, which resolves through a second reaction into two daughter molecules. The two daughter molecules have the same general structure regardless of which pair of sites, attL1 and attR1 or attL2 and attR2, react first to form the co-integrate. The segments change partners by these reactions, regardless of whether the parental molecules are both circular, one is circular and one is linear, or both are linear. In this example, selection for ampicillin resistance carried on the Destination Vector, which also carries the death gene ccdB, provides the means for selecting only for the desired attB product plasmid.

### **Example 3: Protein Expression in the Recombinational Cloning System**

Proteins are expressed *in vivo* as a result of two processes, transcription (DNA into RNA), and translation (RNA into protein). For a review of protein expression in prokaryotes and eukaryotes, see Example 13 below. Many vectors (pUC, BlueScript, pGem) use interruption of a transcribed lacZ gene for blue-white screening. These plasmids, and many Expression Vectors, use the lac promoter to control expression of cloned genes. Transcription from the lac



promoter is turned on by adding the inducer IPTG. However, a low level of RNA is made in the absence of inducer, i.e., the lac promoter is never completely off. The result of this "leakiness" is that genes whose expression is harmful to *E. coli* may prove difficult or impossible to clone in vectors that contain the lac promoter, or they may be cloned only as inactive mutants.

In contrast to other gene expression systems, nucleic acid molecules cloned into an Entry Vector may be designed *not* to be expressed. The presence of the strong transcriptional terminator *rrnB* (Orosz, et al., *Eur. J. Biochem.* 201: 653, 1991) just upstream of the attL1 site keeps transcription from the vector promoters (drug resistance and replication origin) from reaching the cloned gene. However, if a toxic gene is cloned into a Destination Vector, the host may be sick, just as in other expression systems. But the reliability of subcloning by *in vitro* recombination makes it easier to recognize that this has happened -- and easier to try another expression option in accordance with the methods of the invention, if necessary.

#### **Example 4: Choosing the Right Entry Vector**

There are two kinds of choices that must be made in choosing the best Entry Vector, dictated by (1) the particular DNA segment that is to be cloned, and (2) what is to be accomplished with the cloned DNA segment. These factors are critical in the choice of Entry Vector used, because when the desired nucleic acid molecule of interest is moved from the Entry Vector to a Destination Vector, all the base pairs between the nucleic acid molecule of interest and the Int cutting sites in attL1 and attL2 (such as in Figure 6) move into the Destination Vector as well. For genomic DNAs that are not expressed as a result of moving into a Destination Vector, these decisions are not as critical.

For example, if an Entry Vector with certain translation start signals is used, those sequences will be translated into amino acids if an amino-terminal fusion to the desired nucleic acid molecule of interest is made. Whether the desired nucleic acid molecule of interest is to be expressed as fusion protein, native protein, or both, dictates whether translational start sequences must be included between the attB sites of the clone (native protein) or, alternatively, supplied by the Destination

Vector (fusion protein). In particular, Entry Clones that include translational start sequences may prove less suitable for making fusion proteins, as internal initiation of translation at these sites can decrease the yield of N-terminal fusion protein. These two types of expression afforded by the compositions and methods of the invention are illustrated in Figure 62.

No Entry Vector is likely to be optimal for all applications. The nucleic acid molecule of interest may be cloned into any of several optimal Entry Vectors.

As an example, consider pENTR7 (Figure 16) and pENTR11 (Figure 20), which are useful in a variety of applications, including (but not limited to):

- Cloning cDNAs from most of the commercially available libraries. The sites to the left and right of the *ccdB* death gene have been chosen so that directional cloning is possible if the DNA to be cloned does not have two or more of these restriction sites.

- Cloning of genes directionally: *Sall*, *BamHI*, *XmnI* (blunt), or *KpnI* on the left of *ccdB*; *NotI*, *XhoI*, *XbaI*, or *EcoRV* (blunt), on the right.

- Cloning of genes or gene fragments with a blunt amino end at the *XmnI* site. The *XmnI* site has four of the six most favored bases for eukaryotic expression (see Example 13, below), so that if the first three bases of the DNA to be cloned are ATG, the open reading frame (ORF) will be expressed in eukaryotic cells (e.g., mammalian cells, insect cells, yeast cells) when it is transcribed in the appropriate Destination Vector. In addition, in pENTR11, a Shine-Dalgarno sequence is situated 8 bp upstream, for initiating protein synthesis in a prokaryotic host cell (particularly a bacterial cell, such as *E. coli*) at an ATG.

- Cleaving off amino terminal fusions (e.g., His<sub>6</sub>, GST, or thioredoxin) using the highly specific TEV (Tobacco Etch Virus) protease (available from Life Technologies, Inc.). If the nucleic acid molecule of interest is cloned at the

blunt *Xmn*I site, TEV cleavage will leave two amino acids on the amino end of the expressed protein.

• Selecting against uncut or singly cut Entry Vector molecules during cloning with restriction enzymes and ligase. If the *ccdB* gene is not removed with a double digest, it will kill any recipient *E. coli* cell that does not contain a mutation that makes the cell resistant to *ccdB* (see U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, the disclosure of which is incorporated by reference herein in its entirety).

• Allowing production of amino fusions with ORFs in all cloning sites. There are no stop codons (in the *attL1* reading frame) upstream of the *ccdB* gene.

In addition, pENTR11 is also useful in the following applications:

• Cloning cDNAs that have an *Nco*I site at the initiating ATG into the *Nco*I site. Similar to the *Xmn*I site, this site has four of the six most favored bases for eukaryotic expression. Also, a Shine-Dalgarno sequence is situated 8 bp upstream, for initiating protein synthesis in a prokaryotic host cell (particularly a bacterial cell, such as *E. coli*) at an ATG.

• Producing carboxy fusion proteins with ORFs positioned in phase with the reading frame convention for carboxy-terminal fusions (see Figure 20A).

Table 1 lists some non-limiting examples of Entry Vectors and their characteristics, and Figures 10-20 show their cloning sites. All of the Entry Vectors listed in Table 1 are available commercially from Life Technologies, Inc., Rockville, Maryland. Other Entry Vectors not specifically listed here, which comprise alternative or additional features may be made by one of ordinary skill using routine methods of molecular and cellular biology, in view of the disclosure contained herein.

Table 1 Examples of Entry Vectors

Designation	Mnemonic Name	Class of Entry Vector	Distinctive Cloning Sites	Amino Fusions	Native Protein in E.coli	Native Protein in Eukaryotic Cells	Protein Synthesis Features
pENTR-1A, 2B, 3C	Minimal blunt RF A, B, C	Alternative Reading Frame Vectors	Reading frame A, B, or C; blunt cut closest to attL1	Good	Poor	Good	Minimal amino acids between tag and protein; no SD
pENTR4	Minimal Nco	Restr. Enz. Cleavage Vectors	Nco I site (common in euk. cDNAs) closest to attL1	Good	Poor	Good	Good Kozac; no SD
pENTR5	Minimal Nde	Restr. Enz. Cleavage Vectors	NdeI site closest to attL1	Good	Poor	Poor at Nde I, Good at Xmn I	No SD; poor Kozac at Nde, good at Xmn
pENTR6	Minimal Sph	Restr. Enz. Cleavage Vectors	Sph I site closest to attL1	Good	Poor	Poor at Sph I, Good at Xmn I	No SD; poor Kozac at Sph, good at Xmn
pENTR7	TEV Blunt	TEV Cleavage Site Present	Xmn I (blunt) is first cloning site after TEV site	Good	Poor	Good at Xmn I site	TEV protease leaves Gly-Thr on amino end of protein; no SD
pENTR8	TEV Nco	TEV Cleavage Site Present	Nco I is first cloning site after TEV site	Good	Poor	Good	TEV protease leaves Gly-Thr on amino end of protein; no SD

pENTR9	TEV Nde	TEV Cleavage Site Present	Nde I is first cloning site after TEV site	Good	Poor	Poor	TEV protease leaves Gly-Thr on amino end of protein, no SD, poor Kozac
pENTR10	Nde with SD	Good SD for E.coli Expression	Strong SD; Nde I site, no TEV	Poor	Good	Poor	Strong SD, internal starts in amino fusions. Poor Kz. No TEV
pENTR11	2 X SD+Kozac	Good SD for E.coli Expression	Xmn I (blunt) and Nco I sites each preceded by SD and Kozac	Good	Good	Good	Strong SD/Koz Internal starts in amino fusions. No TEV

Entry vectors pENTR1A (Figures 10A and 10B), pENTR2B (Figures 11A and 11B), and pENTR3C (Figures 12A and 12B) are almost identical, except that the restriction sites are in different reading frames. Entry vectors pENTR4 (Figures 13A and 13B), pENTR5 (Figures 14A and 14B), and pENTR6 (Figures 15A and 15B) are essentially identical to pENTR1A, except that the blunt *DraI* site has been replaced with sites containing the ATG methionine codon: *NcoI* in pENTR4, *NdeI* in pENTR5, and *SphI* in pENTR6. Nucleic acid molecules that contain one of these sites at the initiating ATG can be conveniently cloned in these Entry vectors. The *NcoI* site in pENTR4 is especially useful for expression of nucleic acid molecules in eukaryotic cells, since it contains many of the bases that give efficient translation (*see* Example 13, below). (Nucleic acid molecules of interest cloned into the *NdeI* site of pENTR5 are not expected to be highly expressed in eukaryotic cells, because the cytosine at position -3 from the initiating ATG is rare in eukaryotic genes.)

Entry vectors pENTR7 (Figures 16A and 16B), pENTR8 (Figures 17A and 17B), and pENTR9 (Figures 18A and 18B) contain the recognition site for the TEV protease between the attL1 site and the cloning sites. Cleavage sites for *XmnI* (blunt), *NcoI*, and *NdeI*, respectively, are the most 5' sites in these Entry vectors. Amino fusions can be removed efficiently if nucleic acid molecules are cloned into these Entry vectors. TEV protease is highly active and highly specific.

#### ***Example 5: Controlling Reading Frame***

One of the trickiest tasks in expression of cloned nucleic acid molecules is making sure the reading frame is correct. (Reading frame is important if fusions are being made between two ORFs, for example between a nucleic acid molecule of interest and a His6 or GST domain.) For purposes of the present invention, the following convention has been adopted: The reading frame of the DNA cloned into any Entry Vector must be in phase with that of the attB1 site shown in Figure 16A, pENTR7. Notice that the six As of the attL1 site are split into two lysine codons (aaa aaa). The Destination Vectors that make amino fusions were constructed such that they enter the attR1 site in this reading frame.

Destination Vectors for carboxy terminal fusions were also constructed, including those containing His<sub>6</sub> (pDEST23; Figure 43), GST (pDEST24; Figure 44), or thioredoxin (pDEST25; Figure 45) C-terminal fusion sequences.

Therefore, if a nucleic acid molecule of interest is cloned into an Entry Vector so that the aaa aaa reading frame within the attL1 site is in phase with the nucleic acid molecule's ORF, amino terminal fusions will automatically be correctly phased, for all the fusion tags. This is a significant improvement over the usual case, where each different vector can have different restriction sites and different reading frames.

See Example 15 for a practical example of how to choose the most appropriate combinations of Entry Vector and Destination Vector.

#### Materials

Unless otherwise indicated, the following materials were used in the remaining Examples included herein:

#### 5X LR Reaction Buffer:

200-250 mM (preferably 250 mM) Tris-HCl, pH 7.5

250-350 mM (preferably 320 mM) NaCl

1.25-5 mM (preferably 4.75 mM) EDTA

12.5-35 mM (preferably 22-35 mM, and most preferably 35 mM)

Spermidine-HCl

1 mg/ml bovine serum albumin

#### GATEWAY™ LR Clonase™ Enzyme Mix:

per 4 µl of 1X LR Reaction Buffer:

150 ng carboxy-His6-tagged Int (see U.S. Appl. Nos. 60/108,324, filed November 13, 1998, and 09/438,358, filed November 12, 1999, both entirely incorporated by reference herein)

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25 ng carboxy-His6-tagged Xis (see U.S. Appl. Nos. 60/108,324, filed  
November 13, 1998, and 09/438,358, filed November 12,  
1999, both entirely incorporated by reference herein)

30 ng IHF

50% glycerol

**5X BP Reaction Buffer:**

125 mM Tris-HCl, pH 7.5

110 mM NaCl

25 mM EDTA

25 mM Spermidine-HCl

5 mg/ml bovine serum albumin

**GATEWAY™ BP Clonase™ Enzyme Mix:**

per 4 µl of 1X BP Reaction Buffer:

200 ng carboxy-His6-tagged Int (see U.S. Appl. Nos. 60/108,324, filed  
November 13, 1998, and 09/438,358, filed November 12,  
1999, both entirely incorporated by reference herein)

80 ng IHF

50% glycerol

**10X Clonase Stop Solution:**

50 mM Tris-HCl, pH 8.0

1 mM EDTA

2 mg/ml Proteinase K

**Example 6: LR ("Destination") Reaction**

To create a new Expression Clone containing the nucleic acid molecule of  
interest (and which may be introduced into a host cell, ultimately for production  
of the polypeptide encoded by the nucleic acid molecule), an Entry Clone or  
Vector containing the nucleic acid molecule of interest, prepared as described



herein, is reacted with a Destination Vector. In the present example, a  $\beta$ -Gal gene flanked by attL sites is transferred from an Entry Clone to a Destination Vector.

Materials needed:

- 5 X LR Reaction buffer
- Destination Vector (preferably linearized), 75-150 ng/ $\mu$ l
- Entry Clone containing nucleic acid molecule of interest, 100-300 ng in  $\leq 8 \mu$ l TE buffer
- Positive control Entry Clone (pENTR- $\beta$ -Gal) DNA (See note, below)
- Positive control Destination Vector, pDEST1 (pTrc), 75 ng/ $\mu$ l
- GATEWAY™ LR Clonase™ Enzyme Mix (stored at - 80° C)
- 10X Clonase Stop solution
- pUC19 DNA, 10 pg/ $\mu$ l
- Chemically competent *E. coli* cells (competence:  $\geq 1 \times 10^7$  CFU/ $\mu$ g), 400  $\mu$ l.
- LB Plates containing ampicillin (100  $\mu$ g/ml) and methicillin (200  $\mu$ g/ml)  $\pm$  X-gal and IPTG (See below)

Notes:

Preparation of the Entry Clone DNA: Miniprep DNA that has been treated with RNase works well. A reasonably accurate quantitation ( $\pm 50\%$ ) of the DNA to be cloned is advised, as the GATEWAY™ reaction appears to have an optimum of about 100-300 ng of Entry Clone per 20  $\mu$ l of reaction mix.

The positive control Entry Clone, pENTR- $\beta$ -Gal, permits functional analysis of clones based on the numbers of expected blue vs. white colonies on LB plates containing IPTG + Blue-gal (or X-gal), in addition to ampicillin (100  $\mu$ g/ml) and methicillin (200  $\mu$ g/ml). Because  $\beta$ -Galactosidase is a large protein, it often yields a less prominent band than many smaller proteins do on SDS protein gels.

In the Positive Control Entry Vector pENTR- $\beta$ -Gal, the coding sequence of  $\beta$ -Gal has been cloned into pENTR11 (Figures 20A and 20B), with translational start signals permitting expression in *E. coli*, as well as in eukaryotic

cells. The positive control Destination Vector, for example pDEST1 (Figure 21), is preferably linearized.

To prepare X-gal + IPTG plates, either of the following protocols may be used:

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A. With a glass rod, spread over the surface of an LB agar plate: 40  $\mu$ l of 20 mg/ml X-gal (or Bluo-gal) in DMF plus 4  $\mu$ l 200 mg/ml IPTG. Allow liquid to adsorb into agar for 3-4 hours at 37° C before plating cells.

10

B. To liquid LB agar at ~45°C, add: X-gal (or Bluo-Gal) (20 mg/ml in DMF) to make 50  $\mu$ g/ml and IPTG (200 mM in water) to make 0.5-1 mM, just prior to pouring plates. Store X-gal and Bluo-Gal in a light-shielded container.

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Colony color may be enhanced by placing the plates at 5° C for a few hours after the overnight incubation at 37° C. Protocol B can give more consistent colony color than A, but A is more convenient when selection plates are needed on short notice.

20

Recombination in Clonase reactions continues for many hours. While incubations of 45-60 minutes are usually sufficient, reactions with large DNAs, or in which both parental DNAs are supercoiled, or which will be transformed into cells of low competence, can be improved with longer incubation times, such as 2-24 hours at 25° C.

#### Procedure:

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1. Assemble reactions as follows (combine all components at room temperature, except GATEWAY™ LR Clonase™ Enzyme Mix ("Clonase LR"), before removing Clonase LR from frozen storage):

Component	Tube 1	Tube 2	Tube 3	Tube 4
	Neg.	Pos.	Neg.	Test
p-Gate- $\beta$ Gal, (Positive control Entry Clone) 75 ng/ $\mu$ l	4 $\mu$ l	4 $\mu$ l		
pDEST1 (Positive control Destination Vector), 75 ng/ $\mu$ l	4 $\mu$ l	4 $\mu$ l		
Your Entry Clone (100-300 ng)			1 - 8 $\mu$ l	1 - 8 $\mu$ l
Destination Vector for your nucleic acid molecule, 75 ng/ $\mu$ l			4 $\mu$ l	4 $\mu$ l
5 X LR Reaction Buffer	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l
TE	8 $\mu$ l	4 $\mu$ l	To 20 $\mu$ l	To 16 $\mu$ l
GATEWAY™ LR Clonase™ Enzyme Mix (store at -80° C, add last)	---	4 $\mu$ l	---	4 $\mu$ l
Total Volume	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l

2. Remove the GATEWAY™ LR Clonase™ Enzyme Mix from the -80° C freezer, place immediately on ice. The Clonase takes only a few minutes to thaw.
3. Add 4  $\mu$ l of GATEWAY™ LR Clonase™ Enzyme Mix to reactions #2 and #4;
4. Return GATEWAY™ LR Clonase™ Enzyme Mix to -80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes.
6. Add 2  $\mu$ l Clonase Stop solution to all reactions. Incubate for 20 min at 37°C. (This step usually increases the total number of colonies obtained by 10-20 fold.)
7. Transform 2  $\mu$ l into 100  $\mu$ l competent *E. coli*. Select on plates containing ampicillin at 100  $\mu$ g/ml.

#### **Example 7: Transformation of *E. coli***

To introduce cloning or Expression Vectors prepared using the recombinational cloning system of the invention, any standard *E. coli* transformation protocol should be satisfactory. The following steps are recommended for best results:

1. Let the mixture of competent cells and Recombinational Cloning System reaction product stand on ice at least 15 minutes prior to the heat-shock step. This gives time for the recombination proteins to dissociate from the DNA, and improves the transformation efficiency.

2. Expect the reaction to be about 1%-5% efficient, i.e., 2  $\mu$ l of the reaction should contain at least 100 pg of the Expression Clone plasmid (taking into account the amounts of each parental plasmid in the reaction, and the subsequent dilution). If the E. coli cells have a competence of  $10^7$  CFU/ $\mu$ g, 100 pg of the desired clone plasmid will give about 1000 colonies, or more, if the entire transformation is spread on one ampicillin plate.

3. Always do a control pUC DNA transformation. If the number of colonies is not what you expect, the pUC DNA transformation gives you an indication of where the problem was.

***Example 8: Preparation of attB-PCR Product***

For preparation of attB-PCR products in the PCR cloning methods described in Example 9 below, PCR primers containing attB1 and attB2 sequences are used. The attB1 and attB2 primer sequences are as follows:

**attB1:** 5'-GGGGACAAGTTTGTACAAAAAGCAGGCT-(template-specific sequence)-3'

**attB2:** 5'-GGGGACCACTTTGTACAAGAAAGCTGGGT-(template-specific sequence)-3'

The attB1 sequence should be added to the amino primer, and the attB2 sequence to the carboxy primer. The 4 guanines at the 5' ends of each of these primers enhance the efficiency of the minimal 25 bp attB sequences as substrates for use in the cloning methods of the invention.

Standard PCR conditions may be used to prepare the PCR product. The following suggested protocol employs PLATNUM *Taq* DNA Polymerase High

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Fidelity®, available commercially from Life Technologies, Inc. (Rockville, MD). This enzyme mix eliminates the need for hot starts, has improved fidelity over Taq, and permits synthesis of a wide range of amplicon sizes, from 200 bp to 10 kb, or more, even on genomic templates.

#### Materials needed:

- PLATINUM Taq DNA Polymerase High Fidelity® (Life Technologies, Inc.)
- attB1- and attB2- containing primer pair (see above) specific for your template
- DNA template (linearized plasmid or genomic DNA)
- 10X High Fidelity PCR Buffer
- 10 mM dNTP mix
- PEG/MgCl<sub>2</sub> Mix (30% PEG 8000, 30 mM MgCl<sub>2</sub>)

#### Procedure:

1.) Assemble the reaction as follows:

Component	Reaction with Plasmid Target	Reaction with <u>Genomic</u> Target
10X High Fidelity PCR Buffer	5 µl	5 µl
dNTP Mix 10 mM	1 µl	1 µl
MgSO <sub>4</sub> , 50mM	2 µl	2 µl
attB1 Primer, 10 µM	2 µl	1 µl
attB2 Primer, 10 µM	2 µl	1 µl
Template DNA	1-5 ng*	≥ 100 ng
PLATINUM Taq High Fidelity	2 µl	1 µl
Water	to 50 µl	to 50 µl

\* Use of higher amounts of plasmid template may permit fewer cycles (10-15) of PCR

2.) Add 2 drops mineral oil, as appropriate.

3.) Denature for 30 sec. at 94°C.

4.) Perform 25 cycles:

94°C for 15 sec-30 sec

55°C for 15 sec-30 sec

68°C for 1 min per kb of template.

5.) Following the PCR reaction, apply 1-2 µl of the reaction mixture to an agarose gel, together with size standards (e.g., 1 Kb Plus Ladder, Life Technologies, Inc.) and quantitation standards (e.g., Low Mass Ladder, Life Technologies, Inc.), to assess the yield and uniformity of the product.

Purification of the PCR product is recommended, to remove attB primer dimers which can clone efficiently into the Entry Vector. The following protocol is fast and will remove DNA <300 bp in size:

6.) Dilute the 50 µl PCR reaction to 200 µl with TE.

7.) Add 100 µl PEG/MgCl<sub>2</sub> Solution. Mix and centrifuge immediately at 13,000 RPM for 10 min at room temperature. Remove the supernatant (pellet is clear and hard to see).

8.) Dissolve the pellet in 50 µl TE and check recovery on a gel.

If the starting PCR template is a plasmid that contains the gene for Kan<sup>r</sup>, it is advisable to treat the completed PCR reaction with the restriction enzyme *DpnI*, to degrade the plasmid since unreacted residual starting plasmid is a potential source of false-positive colonies from the transformation of the GATEWAY™ Cloning System reaction. Adding ~5 units of *DpnI* to the completed PCR reaction and incubating for 15 min at 37°C will eliminate this potential problem. Heat inactivate the *DpnI* at 65°C for 15 min, prior to using the PCR product in the GATEWAY™ Cloning System reaction.

**Example 9: Cloning attB-PCR products into Entry Vectors via the BP ("Gateway") Reaction**

The addition of 5'-terminal attB sequences to PCR primers allows synthesis of a PCR product that is an efficient substrate for recombination with a Donor (attP) Plasmid in the presence of GATEWAY™ BP Clonase™ Enzyme Mix. This reaction produces an Entry Clone of the PCR product (See Figure 8).

The conditions of the Gateway Cloning reaction with an attB PCR substrate are similar to those of the BP Reaction (see Example 10 below), except that the attB-PCR product (see Example 8) substitutes for the Expression Clone, and the attB-PCR positive control (attB-tet<sup>r</sup>) substitutes for the Expression Clone Positive Control (GFP).

Materials needed:

- 5 X BP Reaction Buffer
- Desired attB-PCR product DNA, 50-100 ng in  $\leq 8 \mu\text{l}$  TE.
- Donor (attP) Plasmid (Figures 49-54), 75 ng/ $\mu\text{l}$ , supercoiled DNA
- attB-tet<sup>r</sup> PCR product positive control, 25 ng/ $\mu\text{l}$
- GATEWAY™ BP Clonase™ Enzyme Mix (stored at - 80° C)
- 10x Clonase Stop Solution
- pUC19 DNA, 10 pg/ $\mu\text{l}$ .
- Chemically competent E.coli cells (competence:  $\geq 1 \times 10^7$  CFU/ $\mu\text{g}$ ), 400  $\mu\text{l}$

Notes:

- Preparation of attB-PCR DNA: see Example 8.
- The Positive Control attB-tet<sup>r</sup> PCR product contains a functional copy of the tet<sup>r</sup> gene of pBR322, with its own promoter. By plating the transformation of the control BP Reaction on kanamycin (50  $\mu\text{g/ml}$ ) plates (if kan<sup>r</sup> Donor Plasmids are used; see Figures 49-52) or an alternative selection agent (e.g., gentamycin, if gen<sup>r</sup> Donor Plasmids are used; see Figure 54), and then picking about 50 of these colonies onto plates with tetracycline (20  $\mu\text{g/ml}$ ), the

percentage of Entry Clones containing functional tet<sup>r</sup> among the colonies from the positive control reaction can be determined (% Expression Clones = (number of tet<sup>r</sup> + kan<sup>r</sup> (or gen<sup>r</sup>) colonies/kan<sup>r</sup> (or gen<sup>r</sup>) colonies).

### **Procedure:**

1. Assemble reactions as follows. Combine all components except GATEWAY™ BP Clonase™ Enzyme Mix, before removing GATEWAY™ BP Clonase™ Enzyme Mix from frozen storage.

	Neg.	Pos.	Test
Component	Tube 1	Tube 2	Tube 3
attB-PCR product, 50-100 ng			1 - 8 µl
Donor (attP) Plasmid 75 ng/µl	2 µl	2 µl	2 µl
attB-PCR tet <sup>r</sup> control DNA (75 ng/µl)		4 µl	
5 X BP Reaction Buffer	4 µl	4 µl	4 µl
TE	10 µl	6 µl	To 16 µl
GATEWAY™ BP Clonase™ Enzyme Mix (store at -80° C, add last)	4 µl	4 µl	4 µl
Total Volume	20 µl	20 µl	20 µl

2. Remove the GATEWAY™ BP Clonase™ Enzyme Mix from the -80° C freezer, place immediately on ice. The Clonase takes only a few minutes to thaw.
3. Add 4 µl of GATEWAY™ BP Clonase™ Enzyme Mix to the subcloning reaction, mix.
4. Return GATEWAY™ BP Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes.



6. Add 2  $\mu$ l Proteinase K (2  $\mu$ g/ $\mu$ l) to all reactions. Incubate for 20 min at 37°C.
7. Transform 2  $\mu$ l into 100  $\mu$ l competent *E. coli*, as per 3.2, above. Select on LB plates containing kanamycin, 50  $\mu$ g/ml.

#### Results:

In initial experiments, primers for amplifying tetR and ampR from pBR322 were constructed containing only the tetR- or ampR-specific targeting sequences, the targeting sequences plus attB1 (for forward primers) or attB2 (for reverse primers) sequences shown in Figure 9, or the attB1 or attB2 sequences with a 5' tail of four guanines. The construction of these primers is depicted in Figure 65. After PCR amplification of tetR and ampR from pBR322 using these primers and cloning the PCR products into host cells using the recombinational cloning system of the invention, the results shown in Figure 66 were obtained. These results demonstrated that primers containing attB sequences provided for a somewhat higher number of colonies on the tetracycline and ampicillin plates. However, inclusion of the 5' extensions of four or five guanines on the primers in addition to the attB sequences provided significantly better cloning results, as shown in Figures 66 and 67. These results indicate that the optimal primers for cloning of PCR products using recombinational cloning will contain the recombination site sequences with a 5' extension of four or five guanine bases.

To determine the optimal stoichiometry between attB-containing PCR products and attP-containing Donor plasmid, experiments were conducted where the amount of PCR product and Donor plasmid were varied during the BP Reaction. Reaction mixtures were then transformed into host cells and plated on tetracycline plates as above. Results are shown in Figure 68. These results indicate that, for optimal recombinational cloning results with a PCR product in the size range of the tet gene, the amounts of attP-containing Donor plasmids are between about 100-500 ng (most preferably about 200-300 ng), while the optimal concentrations of attB-containing PCR products is about 25-100 ng (most preferably about 100 ng), per 20  $\mu$ l reaction.

Experiments were then conducted to examine the effect of PCR product size on efficiency of cloning via the recombinational cloning approach of the invention.

PCR products containing attB1 and attB2 sites, at sizes 256 bp, 1 kb, 1.4 kb, 3.4 kb, 4.6 kb, 6.9 kb and 10.1 kb were prepared and cloned into Entry vectors as described above, and host cells were transformed with the Entry vectors containing the cloned PCR products. For each PCR product, cloning efficiency was calculated relative to cloning of pUC19 positive control plasmids as follows:

$$\text{Cloning Efficiency} = \frac{\text{CFU/ng attB PCR product}}{\text{CFU/ng pUC19 control}} \times \frac{\text{Size (kb) PCR product}}{\text{Size (kb) pUC19 control}}$$

The results of these experiments are depicted in Figures 69A-69C (for 256 bp PCR fragments), 70A-70C (for 1 kb PCR fragments), 71A-71C (for 1.4 kb PCR fragments), 72A-72C (for 3.4 kb PCR fragments), 73A-73C (for 4.6 kb PCR fragments), 74 (for 6.9 kb PCR fragments), and 75-76 (for 10.1 kb PCR fragments). The results shown in these figures are summarized in Figure 77, for different weights and moles of input PCR DNA.

Together, these results demonstrate that attB-containing PCR products ranging in size from about 0.25 kb to about 5 kb clone relatively efficiently in the recombinational cloning system of the invention. While PCR products larger than about 5 kb clone less efficiently (apparently due to slow resolution of cointegrates), longer incubation times during the recombination reaction appears to improve the efficiency of cloning of these larger PCR fragments. Alternatively, it may also be possible to improve efficiency of cloning of large (> about 5 kb) PCR fragments by using lower levels of input attP Donor plasmid and perhaps attB-containing PCR product, and/or by adjusting reaction conditions (*e.g.*, buffer conditions) to favor more rapid resolution of the cointegrates.

#### ***Example 10: The BP Reaction***

One purpose of the Gateward ("Entry") reaction is to convert an Expression Clone into an Entry Clone. This is useful when you have isolated an individual Expression Clone from an Expression Clone cDNA library, and you wish to transfer the nucleic acid molecule of interest into another Expression Vector, or

to move a population of molecules from an attB or attL library. Alternatively, you may have mutated an Expression Clone and now wish to transfer the mutated nucleic acid molecule of interest into one or more new Expression Vectors. In both cases, it is necessary first to convert the nucleic acid molecule of interest to an Entry Clone.

**Materials needed:**

- 5 X BP Reaction Buffer
- Expression Clone DNA, 100-300 ng in  $\leq 8 \mu\text{l}$  TE.
- Donor (attP) Vector, 75 ng/ $\mu\text{l}$ , supercoiled DNA
- Positive control attB-tet-PCR DNA, 25 ng/ $\mu\text{l}$
- GATEWAY™ BP Clonase™ Enzyme Mix (stored at  $-80^{\circ}\text{C}$ )
- Clonase Stop Solution (Proteinase K, 2  $\mu\text{g}/\mu\text{l}$ ).

**Notes:**

Preparation of the Expression Clone DNA: Miniprep DNA treated with RNase works well.

1. As with the LR Reaction (see Example 14), the BP Reaction is strongly influenced by the topology of the reacting DNAs. In general, the reaction is most efficient when one of the DNAs is linear and the other is supercoiled, compared to reactions where the DNAs are both linear or both supercoiled. Further, linearizing the attB Expression Clone (anywhere within the vector) will usually give more colonies than linearizing the Donor (attP) Plasmid. If finding a suitable cleavage site within your Expression Clone vector proves difficult, you may linearize the Donor (attP) Plasmid between the attP1 and attP2 sites (for example, at the *NcoI* site), avoiding the *ccdB* gene. Maps of Donor (attP) Plasmids are given in Figures 49-54.

**Procedure:**

1. Assemble reactions as follows. Combine all components at room temperature, except GATEWAY™ BP Clonase™ Enzyme Mix, before removing GATEWAY™ BP Clonase™ Enzyme Mix from freezer.

	Neg.	Pos.	Test
Component	Tube 1	Tube 2	Tube 3
Positive Control, attB-tet-PCR DNA, 25 ng/ $\mu$ l	4 $\mu$ l	4 $\mu$ l	
Desired attB Expression Clone DNA (100ng) linearized			1 - 8 $\mu$ l
Donor (attP) Plasmid, 75 ng/ $\mu$ l	2 $\mu$ l	2 $\mu$ l	2 $\mu$ l
5 X BP Reaction Buffer	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l
TE	10 $\mu$ l	6 $\mu$ l	To 16 $\mu$ l
GATEWAY™ BP Clonase™ Enzyme Mix (store at - 80° C, add last)	---	4 $\mu$ l	4 $\mu$ l
Total Volume	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l

2. Remove the GATEWAY™ BP Clonase™ Enzyme Mix from the -80°C freezer, place immediately on ice. The mixture takes only a few minutes to thaw.
3. Add 4  $\mu$ l of GATEWAY™ BP Clonase™ Enzyme Mix to the subcloning reaction, mix.
4. Return GATEWAY™ BP Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes. If both the attB and attP DNAs are supercoiled, incubation for 2-24 hours at 25°C is recommended.
6. Add 2  $\mu$ l Clonase Stop Solution. Incubate for 10 min at 37°C.
7. Transform 2  $\mu$ l into 100  $\mu$ l competent E. coli, as above. Select on LB plates containing 50  $\mu$ g/ml kanamycin.

#### ***Example 11: Cloning PCR Products into Entry Vectors using Standard Cloning Methods***

##### **Preparation of Entry Vectors for Cloning of PCR Products**

All of the Entry Vectors of the invention contain the death gene ccdB as a stuffer between the "left" and "right" restriction sites. The advantage of this arrangement is that there is virtually no background from vector that has not been cut with both restriction enzymes, because the presence of the ccdB gene will kill

all standard *E. coli* strains. Thus it is necessary to cut each Entry Vector twice, to remove the *ccdB* fragment.

We strongly recommend that, after digestion of the Entry Vector with the second restriction enzyme, you treat the reaction with phosphatase (calf intestine alkaline phosphatase, CIAP or thermosensitive alkaline phosphatase, TSAP). The phosphatase can be added directly to the reaction mixture, incubated for an additional time, and inactivated. This step dephosphorylates both the vector and *ccdB* fragments, so that during subsequent ligation there is less competition between the *ccdB* fragment and the DNA of interest for the termini of the Entry Vector.

#### Blunt Cloning of PCR products

Generally PCR products do not have 5' phosphates (because the primers are usually 5' OH), and they are not necessarily blunt. (On this latter point, see Brownstein, et al., *BioTechniques* 20: 1006, 1996 for a discussion of how the sequence of the primers affects the addition of single 3' bases.) The following protocol repairs these two defects.

In a 0.5 ml tube, ethanol precipitate about 40 ng of PCR product (as judged from an agarose gel).

1. Dissolve the precipitated DNA in 10  $\mu$ l comprising 1  $\mu$ l 10 mM rATP, 1  $\mu$ l mixed 2 mM dNTPs (i.e., 2 mM each dATP, dCTP, dTTP, and dGTP), 2  $\mu$ l 5x T4 polynucleotide kinase buffer (350 mM Tris HCl (pH7.6), 50 mM  $MgCl_2$ , 500mM KCl, 5 mM 2-mercaptoethanol) 10 units T4 polynucleotide kinase, 1  $\mu$ l T4 DNA polymerase, and water to 10  $\mu$ l.
2. Incubate the tube at 37° for 10 minutes, then at 65° for 15 minutes, cool, centrifuge briefly to bring any condensate to the tip of the tube.
3. Add 5  $\mu$ l of the PEG/ $MgCl_2$  solution, mix and centrifuge at room temperature for 10 minutes. Discard supernatant.
4. Dissolve the invisible precipitate in 10  $\mu$ l containing 2  $\mu$ l 5x T4 DNA ligase buffer (Life Technologies, Inc.), 0.5 units T4 DNA ligase, and about 50 ng of blunt, phosphatase-treated Entry Vector.

5. Incubate at 25° for 1 hour, then 65° for 10 minutes. Add 90 µl TE, transform 10 µl into 50 - 100 µl competent *E. coli* cells.
6. Plate on kanamycin.

5 **Note:** In the above protocol, steps b-c simultaneously polish the ends of the PCR product (through the exonuclease and polymerase activities of T4 DNA polymerase) and phosphorylate the 5' ends (using T4 polynucleotide kinase). It is necessary to inactivate the kinase, so that the blunt, dephosphorylated vector in step e cannot self ligate. Step d (the PEG precipitation) removes all small  
10 molecules (primers, nucleotides), and has also been found to improve the yield of cloned PCR product by 50 fold.

#### Cloning PCR Products after Digestion with Restriction Enzymes

Efficient cloning of PCR products that have been digested with restriction  
15 enzymes includes three steps: inactivation of *Taq* DNA polymerase, efficient restriction enzyme cutting, and removal of small DNA fragments.

*Inactivation of Taq DNA Polymerase:* Carryover of *Taq* DNA polymerase and dNTPs into a RE digestion significantly reduces the success in cloning a PCR product (D. Fox et al., *FOCUS* 20(1):15, 1998), because *Taq* DNA polymerase  
20 can fill in sticky ends and add bases to blunt ends. Either TAQQUENCH™ (obtainable from Life Technologies, Inc.; Rockville, Maryland) or extraction with phenol can be used to inactivate the *Taq*.

*Efficient Restriction Enzyme Cutting:* Extra bases on the 5' end of each PCR primer help the RE cut near ends of PCR products. With the availability of  
25 cheap primers, adding 6 to 9 bases on the 5' sides of the restriction sites is a good investment to ensure that most of the ends are digested. Incubation of the DNA with a 5-fold excess of restriction enzyme for an hour or more helps ensure success.

*Removal of Small Molecules before Ligation:* Primers, nucleotides,  
30 primer dimers, and small fragments produced by the restriction enzyme digestion,

can all inhibit or compete with the desired ligation of the PCR product to the cloning vector. This protocol uses PEG precipitation to remove small molecules.

Protocol for cutting the ends of PCR products with restriction enzyme(s):

5

1. Inactivation of Taq DNA polymerase in the PCR product:

Option A: Extraction with Phenol

10

A1. Dilute the PCR reaction to 200  $\mu$ l with TE. Add an equal volume of phenol:chloroform:isoamyl alcohol, vortex vigorously for 20 seconds, and centrifuge for 1 minute at room temperature. Discard the lower phase.

15

A2. Extract the phenol from the DNA and concentrate as follows. Add an equal volume of 2-butanol (colored red with "Oil Red O" from Aldrich, if desired), vortex briefly, centrifuge briefly at room temperature. Discard the upper butanol phase. Repeat the extraction with 2-butanol. This time the volume of the lower aqueous phase should decrease significantly. Discard the upper 2-butanol phase.

20

A3. Ethanol precipitate the DNA from the aqueous phase of the above extractions. Dissolve in a 200  $\mu$ l of a suitable restriction enzyme (RE) buffer.

Option B: Inactivation with TaqQuench

25

B1. Ethanol precipitate an appropriate amount of PCR product (100 ng to 1  $\mu$ g), dissolve in 200  $\mu$ l of a suitable RE buffer.

B2. Add 2  $\mu$ l TaqQuench.

30

2. Add 10 to 50 units of restriction enzyme and incubate for at least 1 hour. Ethanol precipitate if necessary to change buffers for digestion at the other end of the PCR product.

3. Add ½ volume of the PEG/MgCl<sub>2</sub> mix to the RE digestion. Mix well and immediately centrifuge at room temperature for 10 minutes. Discard the supernatant (pellet is usually invisible), centrifuge again for a few seconds, discard any remaining supernatant.

4. Dissolve the DNA in a suitable volume of TE (depending on the amount of PCR product in the original amplification reaction) and apply an aliquot to an agarose gel to confirm recovery. Apply to the same gel 20-100 ng of the appropriate Entry Vector that will be used for the cloning.

***Example 12: Determining The Expected Size of the GATEWAY™ Cloning Reaction Products***

If you have access to a software program that will electronically cut and splice sequences, you can create electronic clones to aid you in predicting the sizes and restriction patterns of GATEWAY™ Cloning System recombination products.

The cleavage and ligation steps performed by the enzyme Int in the GATEWAY™ Cloning System recombination reactions mimic a restriction enzyme cleavage that creates a 7-bp 5'-end overhang followed by a ligation step that reseals the ends of the daughter molecules. The recombination proteins present in the Clonase cocktails (see Example 19 below) recognize the 15 bp core sequence present within all four types of att sites (in addition to other flanking sequences characteristic of each of the different types of att sites).

By treating these sites in your software program as if they were restriction sites, you can cut and splice your Entry Clones with various Destination Vectors and obtain accurate maps and sequences of the expected results from your GATEWAY™ Cloning System reactions.

***Example 13: Protein Expression***

**Brief Review of Protein Expression**

*Transcription:* The most commonly used promoters in *E. coli* Expression Vectors are variants of the lac promoter, and these can be turned on by adding



IPTG to the growth medium. It is usually good to keep promoters off until expression is desired, so that the host cells are not made sick by the overabundance of some heterologous protein. This is reasonably easy in the case of the lac promoters used in *E. coli*. One needs to supply the *lac I* gene (or its more productive relative, the *lac I<sup>q</sup>* gene) to make *lac* repressor protein, which binds near the promoter and keeps transcription levels low. Some Destination Vectors for *E. coli* expression carry their own *lacI<sup>q</sup>* gene for this purpose. (However, lac promoters are always a little "on," even in the absence of IPTG.)

Controlling transcription in eukaryotic cells is not nearly so straightforward or efficient. The tetracycline system of Bujard and colleagues is the most successful approach, and one of the Destination Vectors (pDEST11; Figure 31) has been constructed to supply this function.

*Translation:* Ribosomes convert the information present in mRNA into protein. Ribosomes scan RNA molecules looking for methionine (AUG) codons, which begin nearly all nascent proteins. Ribosomes must, however, be able to distinguish between AUG codons that code for methionine in the middle of proteins from those at the start. Most often ribosomes choose AUGs that are 1) first in the RNA (toward the 5' end), and 2) have the proper sequence context. In *E. coli* the favored context (first recognized by Shine and Dalgarno, *Eur. J. Biochem.* 57: 221 (1975)) is a run of purines (As and Gs) from five to 12 bases upstream of the initiating AUG, especially AGGAGG or some variant.

In eukaryotes, a survey of translated mRNAs by Kozak (*J. Biol. Chem.* 266: 19867 (1991)) has revealed a preferred sequence context, gcc Acc ATGG, around the initiating methionine, with the A at -3 being most important, and a purine at +4 (where the A of the ATG is +1), preferably a G, being next most influential. Having an A at -3 is enough to make most ribosomes choose the first AUG of an mRNA, in plants, insects, yeast, and mammals. (For a review of initiation of protein synthesis in eukaryotic cells, see: Pain, V.M. *Eur. J. Biochem.* 236:747-771, 1996.)

*Consequences of Translation Signals for GATEWAY™ Cloning System:* First, translation signals (Shine-Dalgarno in *E. coli*, Kozak in eukaryotes) have to be close to the initiating ATG. The attB site is 25 base pairs long. Thus if

translation signals are desired near the natural ATG of the nucleic acid molecule of interest, they must be present in the Entry Clone of that nucleic acid molecule of interest. Also, when a nucleic acid molecule of interest is moved from an Entry Clone to a Destination vector, any translation signals will move along. The result is that the presence or absence of Shine-Dalgarno and/or Kozak sequences in the Entry Clone must be considered, with the eventual Destination Vectors to be used in mind.

Second, although ribosomes choose the 5' ATG most often, internal ATGs are also used to begin protein synthesis. The better the translation context around this internal ATG, the more internal translation initiation will be seen. This is important in the GATEWAY™ Cloning System, because you can make an Entry Clone of your nucleic acid molecule of interest, and arrange to have Shine-Dalgarno and/or Kozak sequences near the ATG. When this cassette is recombined into a Destination Vector that transcribes your nucleic acid molecule of interest, you get native protein. If you want, you can make a fusion protein in a different Destination Vector, since the Shine-Dalgarno and/or Kozak sequences do not contain any stop signals in the same reading frame. However, the presence of these internal translation signals may result in a significant amount of native protein being made, contaminating, and lowering the yield of, your fusion protein. This is especially likely with short fusion tags, like His6.

A good compromise can be recommended. If an Entry Vector like pENTR7 (Figure 16) or pENTR8 (Figure 17) is chosen, the Kozak bases are present for native eukaryotic expression. The context for *E. coli* translation is poor, so the yield of an amino-terminal fusion should be good, and the fusion protein can be digested with the TEV protease to make near-native protein following purification.

*Recommended Conditions for Synthesis of Proteins in E. coli:* When making proteins in *E. coli* it is advisable, at least initially, to incubate your cultures at 30°C, instead of at 37°C. Our experience indicates that proteins are less likely to form aggregates at 30°C. In addition, the yields of proteins from cells grown at 30°C frequently are improved.

The yields of proteins that are difficult to express may also be improved by inducing the cultures in mid-log phase of growth, using cultures begun in the morning from overnight growths, as opposed to harvesting directly from an overnight culture. In the latter case, the cells are preferably in late log or stationary growth, which can favor the formation of insoluble aggregates.

***Example 14: Constructing Destination Vectors from Existing Vectors***

Destination Vectors function because they have two recombination sites, attR1 and attR2, flanking a chloramphenicol resistance (CmR) gene and a death gene, ccdB. The GATEWAY™ Cloning System recombination reactions exchange the entire Cassette (except for a few bases comprising part of the attB sites) for the DNA segment of interest from the Entry Vector. Because attR1, CmR, ccdB gene, and attR2 are contiguous, they can be moved on a single DNA segment. If this Cassette is cloned into a plasmid, the plasmid becomes a Destination Vector. Figure 63 shows a schematic of the GATEWAY™ Cloning System Cassette; attR cassettes in all three reading frames contained in vectors pEYC15101, pEYC15102 and pEYC15103 are shown in Figures 64A, 64B, and 64C, respectively.

The protocol for constructing a Destination Vector is presented below. Keep in mind the following points:

- Destination Vectors must be constructed and propagated in one of the DB strains of *E. coli* (e.g., DB3.1, and particularly *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells) available from Life Technologies, Inc. (and described in detail in U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), because the ccdB death gene will kill any *E. coli* strain that has not been mutated such that it will survive the presence of the ccdB gene.
- If your Destination Vector will be used to make a fusion protein, a GATEWAY™ Cloning System cassette with the correct reading frame must be used. The nucleotide sequences of the ends of the cassettes are shown in Figure 78. The reading frame of the fusion protein domain must

be in frame with the core region of the attR1 site (for an amino terminal fusion) so that the six As are translated into two lysine codons. For a C-terminal fusion protein, translation through the core region of the attR2 site should be in frame with -TAC-AAA-, to yield -Tyr-Lys-.

- Note that each reading frame Cassette has a different unique restriction site between the chloramphenicol resistance and ccdB genes (*Mlu*I for reading frame A, *Bgl*II for reading frame B, and *Xba*I for reading frame C; see Figure 63).
- Most standard vectors can be converted to Destination Vectors, by inserting the Entry Cassette into the MCS of that vector.

#### Protocol for Making a Destination Vector

1. If the vector will make an amino fusion protein, it is necessary to keep the "aaa" triplets in attR1 in phase with the triplets of the fusion protein. Determine which Entry cassette to use as follows:

a.) Write out the nucleotide sequence of the existing vector near the restriction site into which the Entry cassette will be cloned. These must be written in triplets corresponding to the amino acid sequence of the fusion domain.

b.) Draw a vertical line through the sequence that corresponds to the restriction site end, after it has been cut and made blunt, i.e., after filling in a protruding 5' end or polishing a protruding 3' end.

c.) Choose the appropriate reading frame cassette:

- If the coding sequence of the blunt end ends after a complete codon triplet, use the reading frame A cassette. See Figures 78, 79 and 80.

•If the coding sequence of the blunt end ends in a single base, use the reading frame B cassette. See Figures 78, 79 and 81.

•If the coding sequence of the blunt end ends in two bases, use the reading frame C cassette. See Figures 78, 79, 82A-B, and 83A-C.

2. Cut one to five micrograms of the existing plasmid at the position where you wish your nucleic acid molecule of interest (flanked by att sites) to be after the recombination reactions. Note: it is better to remove as many of the MCS restriction sites as possible at this step. This makes it more likely that restriction enzyme sites within the GATEWAY™ Cloning System Cassette will be unique in the new plasmid, which is important for linearizing the Destination Vector (Example 14, below).

3. Remove the 5' phosphates with alkaline phosphatase. While this is not mandatory, it increases the probability of success.

4. Make the end(s) blunt with fill-in or polishing reactions. For example, to 1 µg of restriction enzyme-cut, ethanol-precipitated vector DNA, add:

- i. 20 µl 5x T4 DNA Polymerase Buffer (165 mM Tris-acetate (pH 7.9), 330 mM Na acetate, 50 mM Mg acetate, 500 µg/ml BSA, 2.5 mM DTT)
- ii. 5 µl 10mM dNTP mix
- iii. 1 Unit of T4 DNA Polymerase
- iv. Water to a final volume of 100 µl
- v. Incubate for 15 min at 37°C.

5. Remove dNTPs and small DNA fragments: Ethanol precipitate (add three volumes of room temperature ethanol containing 0.1 M sodium acetate, mix well, immediately centrifuge at room temperature 5 - 10 minutes), dissolve wet precipitate in 200 µl TE, add 100 µl 30% PEG 8000, 30 mM MgCl<sub>2</sub>, mix well,

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immediately centrifuge for 10 minutes at room temperature, discard supernatant, centrifuge again a few seconds, discard any residual liquid.

6. Dissolve the DNA to a final concentration of 10 - 50 ng per microliter. Apply 20 - 100 ng to a gel next to supercoiled plasmid and linear size standards to confirm cutting and recovery. The cutting does not have to be 100% complete, since you will be selecting for the chloramphenicol marker on the Entry cassette.

7. In a 10  $\mu$ l ligation reaction combine 10 - 50 ng vector, 10 - 20 ng of Entry Cassette (Figure 79), and 0.5 units T4 DNA ligase in ligase buffer. After one hour (or overnight, whichever is most convenient), transform 1  $\mu$ l into one of the DB strains of competent *E. coli* cells with a *gyrA462* mutation (See U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), preferably DB3.1, and most preferably *E. coli* LIBRARY EFFICIENCY@DB3.1™ Competent Cells. The *ccdB* gene on the Entry Cassette will kill other strains of *E. coli* that have not been mutated so as to survive the presence of the *ccdB* gene.

8. After expression in SOC medium, plate 10  $\mu$ l and 100  $\mu$ l on chloramphenicol-containing (30  $\mu$ g / ml) plates, incubate at 37° C.

9. Pick colonies, make miniprep DNA. Treat the miniprep with RNase A and store in TE. Cut with the appropriate restriction enzyme to determine the orientation of the Cassette. Choose clones with the attR1 site next to the amino end of the protein expression function of the plasmid.

#### Notes on Using Destination Vectors

- We have found that about ten-fold more colonies result from a GATEWAY™ Cloning System reaction if the Destination Vector is linear or relaxed. If the competent cells you use are highly competent ( $>10^8$  per microgram), linearizing the Destination Vector is less essential.

- The site or sites used for the linearization must be within the Entry Cassette. Sites that cut once or twice within each cassette are shown in Figures 80-82.
- Minipreps of Destination Vectors will work fine, so long as they have been treated with RNase. Since most DB strains are *endA*- (See U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), minipreps can be digested with restriction enzymes without a prior phenol extraction.
- Reading the OD<sub>260</sub> of miniprep DNA is inaccurate unless the RNA and ribonucleotides have been removed, for example, by a PEG precipitation.

***Example 15: Some Options in Choosing Appropriate Entry Vectors and Destination Vectors: An Example***

In some applications, it may be desirable to express a nucleic acid molecule of interest in two forms: as an amino-terminal fusion in *E. coli*, and as a native protein in eukaryotic cells. This may be accomplished in any of several ways:

**Option 1:** Your choices depend on your nucleic acid molecule of interest and the fragment that contains it, as well as the available Entry Vectors. For eukaryotic translation, you need consensus bases according to Kozak (*J. Biol. Chem.* 266:19867, 1991) near the initiating methionine (ATG) codon. All of the Entry Vectors offer this motif upstream of the *XmnI* site (blunt cutter). One option is to amplify your nucleic acid molecule of interest, with its ATG, by PCR, making the amino end blunt and the carboxy end containing the natural stop codon followed by one of the "right side" restriction sites (*EcoRI*, *NotI*, *XhoI*, *EcoRV*, or *XbaI* of the pENTR vectors).

If you know your nucleic acid molecule of interest does not have, for example, an *XhoI* site, you can make a PCR product that has this structure:

Xho I

```

5' ATG nnn nnn --- nnn TAA ctc gag nnn nnn 3'
3' tac nnn nnn --- nnn att gag ctc nnn nnn 5'
  
```

After cutting with *Xho*I, the fragment is ready to clone:

```

5' ATG nnn nnn --- nnn TAA c      3'
3' tac nnn nnn --- nnn att gag ct  5'

```

(If you follow this example, don't forget to put a phosphate on the amino oligo.)

**Option 2:** This PCR product could be cloned into two Entry Vectors to give the desired products, between the *Xmn*I and *Xho*I sites: pENTR1A (Figures 10A, 10B ) or pENTR7 (Figures 16A, 16B). If you clone into pENTR1A, amino fusions will have the minimal number of amino acids between the fusion domain and your nucleic acid molecule of interest, but the fusion cannot be removed with TEV protease. The converse is true of clones in pENTR7, i.e., an amino fusion can be cleaved with TEV protease, at the cost of more amino acids between the fusion and your nucleic acid molecule of interest.

In this example, let us choose to clone our hypothetical nucleic acid molecule of interest into pENTR7, between the *Xmn*I and *Xho*I sites. Once this is accomplished, several optional protocols using the Entry Clone pENTR7 may be followed:

**Option 3:** Since the nucleic acid molecule of interest has been amplified with PCR, it may be desirable to sequence it. To do this, transfer the nucleic acid molecule of interest from the Entry Vector into a vector that has priming sites for the standard sequencing primers. Such a vector is pDEST6 (Figures 26A, 26B). This Destination Vector places the nucleic acid molecule of interest in the opposite orientation to the lac promoter (which is leaky -- see Example 3 above). If the gene product is toxic to *E. coli*, this Destination Vector will minimize its toxicity.

**Option 4:** While the sequencing is going on, you might wish to check the expression of the nucleic acid molecule of interest in, for example, CHO cells, by recombining the nucleic acid molecule of interest into a CMV promoter vector (pDEST7, Figure 27; or pDEST12, Figure 32), or into a baculovirus vector (pDEST8, Figure 28; or pDEST10, Figure 30) for expression in insect cells. Both



of these vectors will transcribe the coding sequence of your nucleic acid molecule of interest, and translate it from the ATG of the PCR product using the Kozak bases upstream of the *Xmn*I site.

**Option 5:** If you wish to purify protein, for example to make antibodies, you can clone the nucleic acid molecule of interest into a His6 fusion vector, pDEST2 (Figure 22). Since the nucleic acid molecule of interest is cloned downstream of the TEV protease cleavage domain of pENTR7 (Figure 16), the amino acid sequence of the protein produced will be:

[----- attB1 -----]      TEV protease

NH2- MSYYHHHHHHGITSLYKKAGFENLYFQ↓ GTM----COOH

The attB site and the restriction sites used to make the Destination and Entry Vectors are translated into the underlined 11 amino acids (GITSLYKKAGF). Cleavage with TEV protease (arrow) leaves two amino acids, GT, on the amino end of the gene product.

See Figure 55 for an example of a nucleic acid molecule of interest, the chloramphenicol acetyl transferase (CAT) gene, cloned into pENTR7 (Figure 16) as a blunt (amino)-*Xho*I (carboxy) fragment, then cloned by recombination into the His6 fusion vector pDEST2 (Figure 22).

**Option 6:** If the His6 fusion protein is insoluble, you may go on and try a GST fusion. The appropriate Destination vector is pDEST3 (Figure 23).

**Option 7:** If you need to make RNA probes and prefer SP6 RNA polymerase, you can make the top strand RNA with your nucleic acid molecule of interest cloned into pSPORT+ (pDEST5 (Figures 25A, 25B)), and the bottom strand RNA with the nucleic acid molecule of interest cloned into pSPORT(-) (pDEST6 (Figures 26A, 26B)). Opposing promoters for T7 RNA polymerase and SP6 RNA polymerase are also present in these clones.

**Option 8:** It is often worthwhile to clone your nucleic acid molecule of interest into a variety of Destination Vectors in the same experiment. For example, if the number of colonies varies widely when the various recombination reactions are transformed into *E. coli*, this may be an indication that the nucleic acid molecule of interest is toxic in some contexts. (This problem is more clearly evident when a positive control gene is used for each Destination Vector.) Specifically, if many more colonies are obtained when the nucleic acid molecule of interest is recombined into pDEST6 than in pDEST5, there is a good chance that leakiness of the lac promoter is causing some expression of the nucleic acid molecule of interest in pSPORT "+" (which is not harmful in pDEST6 because the nucleic acid molecule of interest is in the opposite orientation).

***Example 16: Demonstration of a One-tube Transfer of a PCR Product (or Expression Clone) to Expression Clone via a Recombinational Cloning Reaction***

In the BxP recombination (Entry or Gateward) reaction described herein, a DNA segment flanked by attB1 and attB2 sites in a plasmid conferring ampicillin resistance was transferred by recombination into an attP plasmid conferring kanamycin resistance, which resulted in a product molecule wherein the DNA segment was flanked by attL sites (attL1 and attL2). This product plasmid comprises an "attL Entry Clone" molecule, because it can react with a "attR Destination Vector" molecule via the LxR (Destination) reaction, resulting in the transfer of the DNA segment to a new (ampicillin resistant) vector. In the previously described examples, it was necessary to transform the BxP reaction products into *E. coli*, select kanamycin resistant colonies, grow those colonies in liquid culture, and prepare miniprep DNA, before reacting this DNA with a Destination Vector in an LxR reaction.

The goal of the following experiment was to eliminate the transformation and miniprep DNA steps, by adding the BxP Reaction products directly to an LxR Reaction. This is especially appropriate when the DNA segment flanked by attB sites is a PCR product instead of a plasmid, because the PCR product cannot give

ampicillin-resistant colonies upon transformation, whereas attB plasmids (in general) carry an ampicillin resistance gene. Thus use of a PCR product flanked by attB sites in a BxP Reaction allows one to select for the ampicillin resistance encoded by the desired attB product of a subsequent LxR Reaction.

Two reactions were prepared: Reaction A, negative control, no attB PCR product, (8  $\mu$ l) contained 50 ng pEZC7102 (attP Donor plasmid, confers kanamycin resistance) and 2  $\mu$ l BxP Clonase (22 ng /  $\mu$ l Int protein and 8 ng/ $\mu$ l IHF protein) in BxP buffer (25 mM Tris HCl, pH 7.8, 70 mM KCl, 5 mM spermidine, 0.5 mM EDTA, 250  $\mu$ g / ml BSA). Reaction B (24  $\mu$ l) contained 150 ng pEZC7102, 6  $\mu$ l BxP Clonase, and 120 ng of the attB -tet-PCR product in the same buffer as reaction A. The attB - tet - PCR product comprised the tetracycline resistance gene of plasmid pBR322, amplified with two primers containing either attB1 or attB2 sites, and having 4 Gs at their 5' ends, as described earlier.

The two reactions were incubated at 25°C for 30 minutes. Then aliquots of these reactions were added to new components that comprised LxR Reactions or appropriate controls for the LxR Reaction. Five new reactions were thus produced:

**Reaction 1:** 5  $\mu$ l of reaction A was added to a 5  $\mu$ l LxR Reaction containing 25 ng NcoI-cut pEZC8402 (the attR Destination Vector plasmid) in LxR buffer (37.5 mM Tris HCl, pH 7.7, 16.5 mM NaCl, 35 mM KCl, 5 mM spermidine, 375  $\mu$ g / ml BSA), and 1  $\mu$ l of GATEWAY™ LR Clonase™ Enzyme Mix (total volume of 10  $\mu$ l).

**Reaction 2:** Same as reaction 1, except 5  $\mu$ l of reaction B (positive) were added instead of reaction A (negative).

**Reaction 3:** Same as reaction 2, except that the amounts of Nco-cut pEZC8402 and GATEWAY™ LR Clonase™ Enzyme Mix were doubled, to 50 ng and 2  $\mu$ l, respectively.

**Reaction 4:** Same as reaction 2, except that 25 ng of pEZ11104 (a positive control attL Entry Clone plasmid) were added in addition to the aliquot of reaction B.

**Reaction 5:** Positive control LxR Reaction, containing 25 ng *Nco*I-cut pEZX8402, 25 ng pEZ11104, 37.5 mM Tris HCl pH 7.7, 16.5 mM NaCl, 35 mM KCl, 5 mM spermidine, 375 µg / ml BSA and 1 µl GATEWAY™ LR Clonase™ Enzyme Mix in a total volume of 5 µl.

All five reactions were incubated at 25°C for 30 minutes. Then, 1 µl aliquots of each of the above five reactions, plus 1 µl from the remaining volume of Reaction B, the standard BxP Reaction, were used to transform 50 µl competent DH5α *E. coli*. DNA and cells were incubated on ice for 15 min., heat shocked at 42°C for 45 sec., and 450 µl SOC were added. Each tube was incubated with shaking at 37°C for 60 min. Aliquots of 100 µl and 400 µl of each transformation were plated on LB plates containing either 50 µg/ml kanamycin or 100 µg/ml ampicillin (see Table 2). A transformation with 10 pg of pUC19 DNA (plated on LB-amp<sub>100</sub>) served as a control on the transformation efficiency of the DH5α cells. Following incubation overnight at 37°C, the number of colonies on each plate was determined.

Results of these reactions are shown in Table 2.

**Table 2\***

Reaction No.	1	2	3	4	5	6
	Number of Colonies					
Vol. plated:	Neg. Control BxP Reaction	1X pEZX8402 and LR Clonase™	2X pEZX8402 and LR Clonase™	LxR Reaction with Pos. Control DNA	LxR Reaction alone	BxP Reaction alone
100 µl	2	1	8	9	~1000	~1000
400 µl	5	10	35	62	>2000	>2000
Selection:	Kan	Amp	Amp	Amp	Amp	Kan

\*(Transformation with pUC 19 DNA yielded  $1.4 \times 10^9$  CFU/µg DNA.)

34 of the 43 colonies obtained from Reaction 3 were picked into 2 ml Terrific Broth with 100 µg/ml ampicillin and these cultures were grown overnight, with shaking, at 37°C. 27 of the 34 cultures gave at least moderate growth, and of these 24 were used to prepare miniprep DNA, using the standard protocol. These 24 DNAs were initially analyzed as supercoiled (SC) DNA on a 1% agarose gel to identify those with inserts and to estimate the sizes of the inserts. Fifteen of the 24 samples displayed SC DNA of the size predicted (5553 bp) if **tetx7102** had correctly recombined with **pEJC8402** to yield **tetx8402**. One of these samples contained two plasmids, one of ~5500 bp and a one of ~3500 bp. The majority of the remaining clones were approximately 4100 bp in size.

All 15 of the clones displaying SC DNA of predicted size (~5500 bp) were analyzed by two different double digests with restriction endonucleases to confirm the structure of the expected product: **tetx8402**. (See plasmid maps, Figures 57-59) In one set of digests, the DNAs were treated with Not I and Eco RI, which should cut the predicted product just outside both attB sites, releasing the tet<sup>r</sup> insert on a fragment of 1475 bp. In the second set of digests, the DNAs were digested with *NotI* and with *NruI*. *NruI* cleaves asymmetrically within the subcloned tet<sup>r</sup> insert, and together with *NotI* will release a fragment of 1019 bp.

Of the 15 clones analyzed by double restriction digestion, 14 revealed the predicted sizes of fragments for the expected product.

#### Interpretation:

The DNA components of Reaction B, **pEJC7102** and attB-tet-PCR, are shown in Figure 56. The desired product of BxP Reaction B is **tetx7102**, depicted in Figure 57. The LxR Reaction recombines the product of the BxP Reaction, **tetx7102** (Figure 57), with the Destination Vector, **pEJC8402**, shown in Figure 58. The LxR Reaction with **tetx7102** plus **pEJC8402** is predicted to yield the desired product **tetx8402**, shown in Figure 59.

Reaction 2, which combined the BxP Reaction and LxR Reaction, gave few colonies beyond those of the negative control Reaction. In contrast, Reaction 3, with twice the amount of **pEJC8402** (Figure 58) and LxR Clonase, yielded a

larger number of colonies. These colonies were analyzed further, by restriction digestion, to confirm the presence of expected product. Reaction 4 included a known amount of attL Entry Clone plasmid in the combined BxP-plus-LxR reaction. But reaction 4 yielded only about 1% of the colonies obtained when the same DNA was used in a LxR reaction alone, Reaction 6. This result suggests that the LxR reaction may be inhibited by components of the BxP reaction.

Restriction endonuclease analysis of the products of Reaction 3 revealed that a sizeable proportion of the colonies (14 of the 34 analyzed) contained the desired tet<sup>r</sup> subclone, tetx8402 (Figure 59).

The above results establish the feasibility of performing first a BxP recombination reaction followed by a LxR recombination reaction -- in the same tube -- simply by adding the appropriate buffer mix, recombination proteins, and DNAs to a completed BxP reaction. This method should prove useful as a faster method to convert attB-containing PCR products into different Expression Clones, eliminating the need to isolate first the intermediate attL-PCR insert subclones, before recombining these with Destination Vectors. This may prove especially valuable for automated applications of these reactions.

This same one-tube approach allows for the rapid transfer of nucleic acid molecules contained in attB plasmid clones into new functional vectors as well. As in the above examples, attL subclones generated in a BxP Reaction can be recombined directly with various Destination Vectors in a LxR reaction. The only additional requirement for using attB plasmids, instead of attB-containing PCR products, is that the Destination Vector(s) employed must contain a different selection marker from the one present on the attB plasmid itself and the attP vector.

Two alternative protocols for a one-tube reaction have also proven useful and somewhat more optimal than the conditions described above.

#### Alternative 1:

Reaction buffer contained 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.25 mM EDTA, 2.5 mM spermidine, and 200 µg/ml BSA. After a 16 (or 3) hour incubation of the PCR product (100 ng) + attP Donor plasmid (100 ng) +

GATEWAY™ BP Clonase™ Enzyme Mix + Destination Vector (100 ng), 2 µl of GATEWAY™ LR Clonase™ Enzyme Mix (per 10 µl reaction mix) was added and the mixture was incubated an additional 6 (or 2) hours at 25°C. Stop solution was then added as above and the mixture was incubated at 37°C as above and transformed by electroporation with 1 µl directly into electrocompetent host cells. Results of this series of experiments demonstrated that longer incubation times (16 hours vs. 3 hours for the BP Reaction, 6 hours vs. 2 hours for the LR Reaction) resulted in about twice as many colonies being obtained as for the shorter incubation times. With two independent genes, 10/10 colonies having the correct cloning patterns were obtained.

Alternative 2:

A standard BP Reaction under the reaction conditions described above for Alternative 1 was performed for 2 hours at 25°C. Following the BP Reaction, the following components were added to the reaction mixture in a total volume of 7 µl:

20 mM Tris-HCl, pH 7.5  
100 mM NaCl  
5 µg/ml Xis-His6  
15% glycerol  
~1000 ng of Destination Vector

The reaction mixture was then incubated for 2 hours at 25°C, and 2.5 µl of stop solution (containing 2 µg/ml proteinase K) was added and the mixture was incubated at 37°C for an additional 10 minutes. Chemically competent host cells were then transformed with 2 µl of the reaction mixture, or electrocompetent host cells (e.g., EMax DH10B cells; Life Technologies, Inc.) were electroporated with 2 µl of the reaction mixture per 25-40 µl of cells. Following transformation, mixtures were diluted with SOC, incubated at 37°C, and plated as described above on media selecting for the selection markers on the Destination Vector and the Entry clone (B x P reaction product). Analogous results to those described for Alternative 1 were obtained with these reaction conditions -- a higher level of colonies containing correctly recombined reaction products were observed.

**Example 17: *Demonstration of a One-tube Transfer of a PCR Product (or Expression Clone) to Expression Clone via a Recombinational Cloning Reaction***

5           Single-tube transfer of PCR product DNA or Expression Clones into Expression Clones by recombinational cloning has also been accomplished using a procedure modified from that described in Example 16. This procedure is as follows:

10           •Perform a standard BP (Gateway) Reaction (see Examples 9 and 10) in 20 µl volume at 25°C for 1 hour.

15           •After the incubation is over, take a 10 µl aliquot from the 20 µl total volume and add 1 µl of Proteinase K (2 mg/ml) and incubate at 37°C for 10 minutes. This first aliquot can be used for transformation and gel assay of BP reaction analysis. Plate BP reaction transformation on LB plates with **Kanamycin** (50 µg/ml).

20           •Add the following reagents to the remaining 10 µl aliquot of the BP reaction:

20                 1 µl of 0.75 M NaCl

20                 2 µl of destination vector (150 ng/µl)

20                 4 µl of LR Clonase™ (after thawing and brief mixing)

25           •Mix all reagents well and incubate at 25°C for 3 hours. Stop the reaction at the end of incubation with 1.7 µl of Proteinase K (2 mg/ml) and incubate at 37°C for 10 minutes.

30           •Transform 2 µl of the completed reaction into 100 µl of competent cells. Plate 100 µl and 400 µl on LB plates with **Ampicillin** (100 µg/ml).

**Notes:**

•If your competent cells are less than 10<sup>8</sup> CFU/µg, and you are concerned about getting enough colonies, you can improve the yield several fold by incubating the



BP reaction for 6-20 hours. Electroporation also can yield better colony output than chemical transformation.

•PCR products greater than about 5-6 kb show significantly lower cloning efficiency in the BP reaction. In this case, we recommend using longer incubation times for both BP and LR steps.

•If you want to move your insert gene into several destination vectors simultaneously, then scale up the initial BP reaction volume so that you have a 10 µl aliquot for adding each destination vector.

***Example 18: Optimization of GATEWAY™ Clonase™ Enzyme Compositions***

The enzyme compositions containing Int and IHF (for BP Reactions) were optimized using a standard functional recombinational cloning reaction (a BP reaction) between attB-containing plasmids and attP-containing plasmids, according to the following protocol:

**Materials and Methods:**

***Substrates:***

AttP - supercoiled pDONR201

AttB - linear ~ 1Kb [<sup>3</sup>H]PCR product amplified from pEZC7501

***Proteins:***

IntH6 -- His<sub>6</sub>-carboxy- tagged λ Integrase

IHF -- Integration Host Factor

***Clonase:***

50 ng/µl IntH6 and 20 ng/µl IHF, admixed in 25 mM Tris- HCl (pH 7.5), 22 mM NaCl, 5 mM EDTA, 1 mg/ml BSA, 5 mM Spermidine, and 50% glycerol.

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*Reaction Mixture (total volume of 40  $\mu$ l):*

1000 ng AttP plasmid  
600 ng AttB [ $^3$ H] PCR product  
8  $\mu$ l Clonase (400 ng IntH6, 160 ng IHF) in 25 mM Tris-HCl (pH 7.5),  
22 mM NaCl, 5 mM EDTA, 1 mg/ml BSA, 5 mM Spermidine, 5 mM  
DTT.

Reaction mixture was incubated for 1 hour at 25°C, 4  $\mu$ l of 2  $\mu$ g/ $\mu$ l  
proteinase K was added and mixture was incubated for an additional 20 minutes  
at 37°C. Mixture was then extracted with an equal volume of Phenol/Chloroform/  
Isoamyl alcohol. The aqueous layer was then collected, and 0.1 volumes of 3 M  
sodium acetate and 2 volumes of cold 100% ethanol were added. Tubes were  
then spun in a microcentrifuge at maximum RPM for 10 minutes at room  
temperature. Ethanol was decanted, and pellets were rinsed with 70% ethanol and  
re-centrifuged as above. Ethanol was decanted, and pellets were allowed to air  
dry for 5-10 minutes and then dissolved in 20  $\mu$ l of 33 mM Tris-Acetate (pH 7.8),  
66 mM potassium acetate, 10 mM magnesium acetate, 1 mM DTT, and 1mM  
ATP. 2 units of exonuclease V (e.g., Plasmid Safe; EpiCentre, Inc., Madison, WI)  
was then added, and the mixture was incubated at 37°C for 30 minutes.

Samples were then TCA-washed by spotting 30  $\mu$ l of reaction mixture  
onto a Whatman GF/C filter, washing filters once with 10% TCA + 1% NaPPi for  
10 minutes, three times with 5% TCA for 5 minutes each, and twice with ethanol  
for 5 minutes each. Filters were then dried under a heat lamp, placed into a  
scintillation vial, and counted on a  $\beta$  liquid scintillation counter (LSC).

The principle behind this assay is that, after exonuclease V digestion, only  
double-stranded circular DNA survives in an acid-insoluble form. All DNA  
substrates and products that have free ends are digested to an acid-soluble form  
and are not retained on the filters. Therefore, only the  $^3$ H-labeled attB linear DNA  
which ends up in circular form after both inter- and intramolecular integration is  
complete is resistant to digestion and is recovered as acid-insoluble product.  
Optimal enzyme and buffer formulations in the Clonase compositions therefore are  
those that give the highest levels of circularized  $^3$ H-labeled attB-containing

sequences, as determined by highest cpm in the LSC. Although this assay was designed for optimization of GATEWAY™ BP Clonase™ Enzyme Mix compositions (Int + IHF), the same type of assay may be performed to optimize GATEWAY™ LR Clonase™ Enzyme Mix compositions (Int + IHF + Xis), except that the reaction mixtures would comprise 1000 ng of AttR (instead of AttP) and 600 ng of AttL (instead of AttB), and 40 ng of His<sub>6</sub>-carboxy- tagged Xis (XisH6) in addition to the IntH6 and IHF.

***Example 19: Testing Functionality of Entry and Destination Vectors***

As part of assessment of the functionality of particular vectors of the invention, it is important to functionally test the ability of the vectors to recombine. This assessment can be carried out by performing a recombinational cloning reaction (as schematized in Figures 2, 4, and 5A and 5B, and as described herein and in commonly owned U.S. Application Nos. 08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, the disclosures of all of which are incorporated by reference herein in their entireties), by transforming E. coli and scoring colony forming units. However, an alternative assay may also be performed to allow faster, more simple assessment of the functionality of a given Entry or Destination Vector by agarose gel electrophoresis. The following is a description of such an in vitro assay.

**Materials and Methods:**

Plasmid templates pEJC1301 (Figure 84) and pEJC1313 (Figure 85), each containing a single wild type att site, were used for the generation of PCR products containing attL or attR sites, respectively. Plasmid templates were linearized with *A*laNI, phenol extracted, ethanol precipitated and dissolved in TE to a concentration of 1 ng/μl.

PCR primers (capital letters represent base changes from wildtype):

attL1            gggg agcct gctttttGtacAaa gttggcatta taaaaaagca ttgc  
attL2            gggg agcct gctttCttGtacAaa gttggcatta taaaaaagca ttgc  
attL right        tgttgccggg aagctagagt aa

attR1            gggg Acaag ttTgtaCaaaaaagc tgaacgaga aacgtaaaat  
attR2            gggg Acaag ttTgtaCaaGaaagc tgaacgaga aacgtaaaat  
attR right        ca gacggcatga tgaacctgaa

PCR primers were dissolved in TE to a concentration of 500 pmol/ $\mu$ l. Primer mixes were prepared, consisting of attL1 + attLright primers, attL2 + attLright primers, attR1 + attRright primers, and attR2 + attRright primers, each mix containing 20 pmol/ $\mu$ l of each primer.

PCR reactions:

1  $\mu$ l plasmid template (1 ng)  
1  $\mu$ l primer pairs (20 pmoles of each)  
3  $\mu$ l of H<sub>2</sub>O  
45  $\mu$ l of Platinum PCR SuperMix® (Life Technologies, Inc.)

Cycling conditions (performed in MJ thermocycler):

95°C/2 minutes  
94°C/30 seconds  
25 cycles of 58°C/30 seconds and 72°C/1.5 minutes  
72°C/5 minutes  
5°C/hold

The resulting attL PCR product was 1.5 kb, and the resulting attR PCR product was 1.0 kb.

PCR reactions were PEG/MgCl<sub>2</sub> precipitated by adding 150  $\mu$ l H<sub>2</sub>O and 100  $\mu$ l of 3x PEG/ MgCl<sub>2</sub> solution followed by centrifugation. The PCR products were dissolved in 50  $\mu$ l of TE. Quantification of the PCR product was performed by gel electrophoresis of 1  $\mu$ l and was estimated to be 50-100 ng/ $\mu$ l.

Recombination reactions of PCR products containing attL or attR sites with GATEWAY™ plasmids was performed as follows:

8 µl of H<sub>2</sub>O

2 µl of attL or attR PCR product (100-200 ng)

5 2 µl of GATEWAY™ plasmid (100 ng)

4 µl of 5x Destination buffer

4 µl of GATEWAY™ LR Clonase™ Enzyme Mix

20 µl total volume (the reactions can be scaled down to a 5 µl total volume by adjusting the volumes of the components to about ¼ of those shown above, while keeping the stoichiometries the same).

10 Clonase reactions were incubated at 25°C for 2 hours. 2 µl of proteinase K (2 mg/ml) was added to stop the reaction. 10 µl was then run on a 1 % agarose gel. Positive control reactions were performed by reacting attL1 PCR product (1.0 kb) with attR1 PCR product (1.5 kb) and by similarly reacting attL2 PCR product with attR2 PCR product to observe the formation of a larger (2.5 kb) recombination product. Negative controls were similarly performed by reacting attL1 PCR product with attR2 PCR product and vice versa or reactions of attL PCR product with an attL plasmid, etc.

20 In alternative assays, to test attB Entry vectors, plasmids containing single attP sites were used. Plasmids containing single att sites could also be used as recombination substrates in general to test all Entry and Destination vectors (*i.e.*, those containing attL, attR, attB and attP sites). This would eliminate the need to do PCR reactions.

#### 25 Results:

Destination and Entry plasmids when reacted with appropriate att-containing PCR products formed linear recombinant molecules that could be easily visualized on an agarose gel when compared to control reactions containing no attL or attR PCR product. Thus, the functionality of Destination and Entry vectors constructed according to the invention may be determined either by carrying out the Destination or Entry recombination reactions as depicted in

Figures 2, 4, and 5A and 5B, or more rapidly by carrying out the linearization assay described in this Example.

***Example 20: PCR Cloning Using Universal Adapter-Primers***

As described herein, the cloning of PCR products using the GATEWAY™ PCR Cloning System (Life Technologies, Inc.; Rockville, MD) requires the addition of attB sites (attB1 and attB2) to the ends of gene-specific primers used in the PCR reaction. The protocols described in the preceding Examples suggest that the user add 29 bp (25 bp containing the attB site plus four G residues) to the gene-specific primer. It would be advantageous to high volume users of the GATEWAY™ PCR Cloning System to generate attB-containing PCR product using universal attB adapter-primers in combination with shorter gene-specific primers containing a specified overlap to the adapters. The following experiments demonstrate the utility of this strategy using universal attB adapter-primers and gene-specific primers containing overlaps of various lengths from 6 bp to 18 bp. The results demonstrate that gene-specific primers with overlaps of 10 bp to 18 bp can be used successfully in PCR amplifications with universal attB adapter-primers to generate full-length PCR products. These PCR products can then be successfully cloned with high fidelity in a specified orientation using the GATEWAY™ PCR Cloning System.

**Methods and Results:**

To demonstrate that universal attB adapter-primers can be used with gene-specific primers containing partial attB sites in PCR reactions to generate full-length PCR product, a small 256 bp region of the human hemoglobin cDNA was chosen as a target so that intermediate sized products could be distinguished from full-length products by agarose gel electrophoresis.

The following oligonucleotides were used:

B1-Hgb: GGGG ACA AGT TTG TAC AAA AAA GCA GGC T-5'-Hgb\*  
B2-Hgb: GGGG ACC ACT TTG TAC AAG AAA GCT GGG T-3'-Hgb\*\*

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18B1-Hgb: TG TAC AAA AAA GCA GGC T-5'-Hgb  
 18B2-Hgb: TG TAC AAG AAA GCT GGG T-3'-Hgb  
 15B1-Hgb: AC AAA AAA GCA GGC T-5'-Hgb  
 15B2-Hgb: AC AAG AAA GCT GGG T-3'-Hgb  
 5 12B1-Hgb: AA AAA GCA GGC T-5'-Hgb  
 12B2-Hgb: AG AAA GCT GGG T-3'-Hgb  
 11B1-Hgb: A AAA GCA GGC T-5'-Hgb  
 11B2-Hgb: G AAA GCT GGG T-3'-Hgb  
 10B1-Hgb: AAA GCA GGC T-5'-Hgb  
 10 10B2-Hgb: AAA GCT GGG T-3'-Hgb  
 9B1-Hgb: AA GCA GGC T-5'-Hgb  
 9B2-Hgb: AA GCT GGG T-3'-Hgb  
 8B1-Hgb: A GCA GGC T-5'-Hgb  
 8B2-Hgb: A GCT GGG T-3'-Hgb  
 15 7B1-Hgb: GCA GGC T-5'-Hgb  
 7B2-Hgb: GCT GGG T-3'-Hgb  
 6B1-Hgb: CA GGC T-5'-Hgb  
 6B2-Hgb: CT GGG T-3'-Hgb  
  
 20 attB1 adapter: GGGG ACA AGT TTG TAC AAA AAA GCA GGC T  
 attB2 adapter: GGGG ACC ACT TTG TAC AAG AAA GCT GGG T  
  
 \* -5'-Hgb = GTC ACT AGC CTG TGG AGC AAG A  
 \*\* -3'-Hgb = AGG ATG GCA GAG GGA GAC GAC A

25

The aim of these experiments was to develop a simple and efficient universal adapter PCR method to generate attB containing PCR products suitable for use in the GATEWAY™ PCR Cloning System. The reaction mixtures and thermocycling conditions should be simple and efficient so that the universal adapter PCR method could be routinely applicable to any PCR product cloning application.

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PCR reaction conditions were initially found that could successfully amplify predominately full-length PCR product using gene-specific primers containing 18bp and 15 bp overlap with universal attB primers. These conditions are outlined below:

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10 pmoles of gene-specific primers  
10 pmoles of universal attB adapter-primers  
1 ng of plasmid containing the human hemoglobin cDNA.  
100 ng of human leukocyte cDNA library DNA.  
5  $\mu$ l of 10x PLATINUM Taq HiFi® reaction buffer (Life Technologies, Inc.)  
2  $\mu$ l of 50 mM MgSO<sub>4</sub>  
1  $\mu$ l of 10 mM dNTPs  
0.2  $\mu$ l of PLATINUM Taq HiFi® (1.0 unit)  
H<sub>2</sub>O to 50  $\mu$ l total reaction volume

## Cycling conditions:

25 x  $\left\{ \begin{array}{l} 95^{\circ}\text{C}/5 \text{ min} \\ 94^{\circ}\text{C}/15 \text{ sec} \\ 50^{\circ}\text{C}/30 \text{ sec} \\ 68^{\circ}\text{C}/1 \text{ min} \\ 68^{\circ}\text{C}/5 \text{ min} \\ 5^{\circ}\text{C}/\text{hold} \end{array} \right.$

To assess the efficiency of the method, 2  $\mu$ l (1/25) of the 50  $\mu$ l PCR reaction was electrophoresed in a 3 % Agarose-1000 gel. With overlaps of 12 bp or less, smaller intermediate products containing one or no universal attB adapter predominated the reactions. Further optimization of PCR reaction conditions was obtained by titrating the amounts of gene-specific primers and universal attB adapter-primers. The PCR reactions were set up as outlined above except that the amounts of primers added were:

0, 1, 3 or 10 pmoles of gene-specific primers  
0, 10, 30 or 100 pmoles of adapter-primers



**Cycling conditions:**

25 x	95°C/3 min
	94°C/15 sec
	50°C/45 sec
	68°C/1 min
	68°C/5 min
	5°C/hold

The use of limiting amounts of gene-specific primers (3 pmoles) and excess adapter-primers (30 pmoles) reduced the amounts of smaller intermediate products. Using these reaction conditions the overlap necessary to obtain predominately full-length PCR product was reduced to 12 bp. The amounts of gene-specific and adapter-primers was further optimized in the following PCR reactions:

0, 1, 2 or 3 pmoles of gene-specific primers  
0, 30, 40 or 50 pmoles of adapter-primers

**Cycling conditions:**

25 x	95°C/3 min
	94°C/15 sec
	48°C/1 min
	68°C/1 min
	68°C/5 min
	5°C/hold

The use of 2 pmoles of gene-specific primers and 40 pmoles of adapter-primers further reduced the amounts of intermediate products and generated predominately full-length PCR products with gene-specific primers containing an 11 bp overlap. The success of the PCR reactions can be assessed in any PCR application by performing a no adapter control. The use of limiting amounts of gene-specific primers should give faint or barely visible bands when 1/25 to 1/10 of the PCR reaction is electrophoresed on a standard agarose gel. Addition of the

universal attB adapter-primers should generate a robust PCR reaction with a much higher overall yield of product.

PCR products from reactions using the 18 bp, 15 bp, 12 bp, 11 bp and 10 bp overlap gene-specific primers were purified using the CONCERT® Rapid PCR Purification System (PCR products greater than 500 bp can be PEG precipitated). The purified PCR products were subsequently cloned into an attP containing plasmid vector using the GATEWAY™ PCR Cloning System (Life Technologies, Inc.; Rockville, MD) and transformed into *E. coli*. Colonies were selected and counted on the appropriate antibiotic media and screened by PCR for correct inserts and orientation.

Raw PCR products (unpurified) from the attB adapter PCR of a plasmid clone of part of the human beta-globin (Hgb) gene were also used in GATEWAY™ PCR Cloning System reactions. PCR products generated with the full attB B1/B2-Hgb, the 12B1/B2, 11B1/B2 and 10B1/B2 attB overlap Hgb primers were successfully cloned into the GATEWAY™ pENTR21 attP vector (Figure 49). 24 colonies from each (24 x 4 = 96 total) were tested and each was verified by PCR to contain correct inserts. The cloning efficiency expressed as cfu/ml is shown below:

Primer Used	cfu/ml
Hgb full attB	8,700
Hgb 12 bp overlap	21,000
Hgb 11 bp overlap	20,500
Hgb 10 bp overlap	13,500
GFP control	1,300

Interestingly, the overlap PCR products cloned with higher efficiency than did the full attB PCR product. Presumably, and as verified by visualization on agarose gel, the adapter PCR products were slightly cleaner than was the full attB PCR product. The differences in colony output may also reflect the proportion of PCR product molecules with intact attB sites.

Using the attB adapter PCR method, PCR primers with 12 bp attB overlaps were used to amplify cDNAs of different sizes (ranging from 1 to 4 kb)

from a leukocyte cDNA library and from first strand cDNA prepared from HeLa total RNA. While three of the four cDNAs were able to be amplified by this method, a non-specific amplification product was also observed that under some conditions would interfere with the gene-specific amplification. This non-specific product was amplified in reactions containing the attB adapter-primers alone without any gene-specific overlap primers present. The non-specific amplification product was reduced by increasing the stringency of the PCR reaction and lowering the attB adapter PCR primer concentration.

These results indicate that the adapter-primer PCR approach described in this Example will work well for cloned genes. These results also demonstrate the development of a simple and efficient method to amplify PCR products that are compatible with the GATEWAY™ PCR Cloning System that allows the use of shorter gene-specific primers that partially overlap universal attB adapter-primers. In routine PCR cloning applications, the use of 12 bp overlaps is recommended. The methods described in this Example can thus reduce the length of gene-specific primers by up to 17 residues or more, resulting in a significant savings in oligonucleotide costs for high volume users of the GATEWAY™ PCR Cloning System. In addition, using the methods and assays described in this Example, one of ordinary skill can, using only routine experimentation, design and use analogous primer-adapters based on or containing other recombination sites or fragments thereof, such as *attL*, *attR*, *attP*, *lox*, FRT, etc.

***Example 21: Mutational Analysis of the Bacteriophage Lambda attL and attR Sites: Determinants of att Site Specificity in Site-specific Recombination***

To investigate the determinants of *att* site specificity, the bacteriophage lambda *attL* and *attR* sites were systematically mutagenized. As noted herein, the determinants of specificity have previously been localized to the 7 bp overlap region (TTTATAC, which is defined by the cut sites for the integrase protein and is the region where strand exchange takes place) within the 15 bp core region (GCTTTTTTATACTAA) which is identical in all four lambda *att* sites, *attB*, *attP*, *attL* and *attR*. This core region, however, has not heretofore been systematically

mutagenized and examined to define precisely which mutations produce unique changes in *att* site specificity.

Therefore, to examine the effect of *att* sequence on site specificity, mutant *attL* and *attR* sites were generated by PCR and tested in an *in vitro* site-specific recombination assay. In this way all possible single base pair changes within the 7 bp overlap region of the core *att* site were generated as well as five additional changes outside the 7 bp overlap but within the 15 bp core *att* site. Each *attL* PCR substrate was tested in the *in vitro* recombination assay with each of the *attR* PCR substrates.

### Methods

To examine both the efficiency and specificity of recombination of mutant *attL* and *attR* sites, a simple *in vitro* site-specific recombination assay was developed. Since the core regions of *attL* and *attR* lie near the ends of these sites, it was possible to incorporate the desired nucleotide base changes within PCR primers and generate a series of PCR products containing mutant *attL* and *attR* sites. PCR products containing *attL* and *attR* sites were used as substrates in an *in vitro* reaction with GATEWAY™ LR Clonase™ Enzyme Mix (Life Technologies, Inc.; Rockville, MD). Recombination between a 1.5 kb *attL* PCR product and a 1.0 kb *attR* PCR product resulted in a 2.5 kb recombinant molecule that was monitored using agarose gel electrophoresis and ethidium bromide staining.

Plasmid templates pEZC1301 (Figure 84) and pEZC1313 (Figure 85), each containing a single wild type *attL* or *attR* site, respectively, were used for the generation of recombination substrates. The following list shows primers that were used in PCR reactions to generate the *attL* PCR products that were used as substrates in L x R Clonase reactions (capital letters represent changes from the wild-type sequence, and the underline represents the 7 bp overlap region within the 15 bp core *att* site; a similar set of PCR primers was used to prepare the *attR* PCR products containing matching mutations):

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GATEWAY™ sites (note: attL2 sequence in GATEWAY™ plasmids begins "accca" while the attL2 site in this example begins "agcct" to reflect wild-type attL outside the core region.):

5

attL1: gggg agcct gcttttttGtacAaa gttggcatta taaaaa-  
agca ttgc

10

attL2: gggg agcct gcttttCttGtacAaa gttggcatta taaaaa-  
agca ttgc

Wild-type:

15

attL0: gggg agcct gctttttttatactaa gttggcatta taaaaa-  
agca ttgc

Single base changes from wild-type:

attLT1A: gggg agcct gcttttAttatactaa gttggcatta taaaaa-  
agca ttgc

20

attLT1C: gggg agcct gcttttCttatactaa gttggcatta taaaaa-  
agca ttgc

attLT1G: gggg agcct gcttttGttatactaa gttggcatta taaaaa-  
agca ttgc

25

attLT2A: gggg agcct gcttttAtatactaa gttggcatta taaaaa-  
agca ttgc

30

attLT2C: gggg agcct gcttttCtatactaa gttggcatta taaaaa-  
agca ttgc

attLT2G: gggg agcct gcttttGtatactaa gttggcatta taaaaa-  
aagca ttgc

35

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attLT3A: gggg agcct gcttttttAatactaa gttggcatta taaaa-  
aagca ttgc

5 attLT3C: gggg agcct gcttttttCatactaa gttggcatta taaaa-  
aagca ttgc

10 attLT3G: gggg agcct gcttttttGatactaa gttggcatta taaaa-  
aagca ttgc

15 attLA4C: gggg agcct gcttttttCtactaa gttggcatta taaaa-  
aagca ttgc

attLA4G: gggg agcct gcttttttGtactaa gttggcatta taaaa-  
aagca ttgc

20 attLA4T: gggg agcct gcttttttTtactaa gttggcatta taaaa-  
aagca ttgc

25 attLT5A: gggg agcct gcttttttAaactaa gttggcatta taaaa-  
aagca ttgc

attLT5C: gggg agcct gcttttttCaactaa gttggcatta taaaa-  
aagca ttgc

30 attLT5G: gggg agcct gcttttttGaactaa gttggcatta taaaa-  
aagca ttgc

35 attLA6C: gggg agcct gcttttttatCctaa gttggcatta taaaa-  
aagca ttgc

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attLA6G: gggg agcct gcttttttatGctaa gttggcatta taaaa-  
aagca ttgc

5 attLA6T: gggg agcct gcttttttatTctaa gttggcatta taaaa-  
aagca ttgc

10 attLC7A: gggg agcct gcttttttataAtaa gttggcatta taaaa-  
aagca ttgc

15 attLC7G: gggg agcct gcttttttataGtaa gttggcatta taaaa-  
aagca ttgc

attLC7T: gggg agcct gcttttttataTtaa gttggcatta taaaa-  
aagca ttgc

Single base changes outside of the 7 bp overlap:

20 attL8: gggg agcct Acttttttataactaa gttggcatta taaaa-  
aagca ttgc

25 attL9: gggg agcct gcCtttttataactaa gttggcatta taaaaa-  
agca ttgc

attL10: gggg agcct gcttCtttataactaa gttggcatta taaaaa-  
agca ttgc

30 attL14: gggg agcct gcttttttataacCaa gttggcatta taaaaa-  
agca ttgc

35 attL15: gggg agcct gcttttttataactaG gttggcatta taaaaa-  
agca ttgc

Note: additional vectors wherein the first nine bases are gggg agcca (*i.e.*, substituting an adenine for the thymine in the position immediately preceding the 15-bp core region), which may or may not contain the single base pair substitutions (or deletions) outlined above, can also be used in these experiments.

5

Recombination reactions of *attL*- and *attR*-containing PCR products was performed as follows:

10

8 µl of H<sub>2</sub>O  
2 µl of attL PCR product (100 ng)  
2 µl of attR PCR product (100 ng)  
4 µl of 5x buffer  
4 µl of GATEWAY™ LR Clonase™ Enzyme Mix  
20 µl total volume

15

Clonase reactions were incubated at 25°C for 2 hours.

2 µl of 10X Clonase stop solution (proteinase K, 2 mg/ml) were added to stop the reaction.

10 µl were run on a 1 % agarose gel.

20

### Results

25

Each *attL* PCR substrate was tested in the *in vitro* recombination assay with each of the *attR* PCR substrates. Changes within the first three positions of the 7 bp overlap (TTTATAC) strongly altered the specificity of recombination. These mutant *att* sites each recombined as well as the wild-type, but only with their cognate partner mutant; they did not recombine detectably with any other *att* site mutant. In contrast, changes in the last four positions (TTTATAC) only partially altered specificity; these mutants recombined with their cognate mutant as well as wild-type *att* sites and recombined partially with all other mutant *att* sites except for those having mutations in the first three positions of the 7 bp

30



overlap. Changes outside of the 7 bp overlap were found not to affect specificity of recombination, but some did influence the efficiency of recombination.

Based on these results, the following rules for *att* site specificity were determined:

- Only changes within the 7 bp overlap affect specificity.
- Changes within the first 3 positions strongly affect specificity.
- Changes within the last 4 positions weakly affect specificity.

Mutations that affected the overall efficiency of the recombination reaction were also assessed by this method. In these experiments, a slightly increased (less than 2-fold) recombination efficiency with *attLT1A* and *attLC7T* substrates was observed when these substrates were reacted with their cognate *attR* partners. Also observed were mutations that decreased recombination efficiency (approximately 2-3 fold), including *attLA6G*, *attL14* and *attL15*. These mutations presumably reflect changes that affect Int protein binding at the core *att* site.

The results of these experiments demonstrate that changes within the first three positions of the 7 bp overlap (TTTATAC) strongly altered the specificity of recombination (*i.e.*, *att* sequences with one or more mutations in the first three thymidines would only recombine with their cognate partners and would not cross-react with any other *att* site mutation). In contrast, mutations in the last four positions (TTTATAC) only partially altered specificity (*i.e.*, *att* sequences with one or more mutations in the last four base positions would cross-react partially with the wild-type *att* site and all other mutant *att* sites, except for those having mutations in one or more of the first three positions of the 7 bp overlap). Mutations outside of the 7 bp overlap were not found to affect specificity of recombination, but some were found to influence (*i.e.*, to cause a decrease in) the efficiency of recombination.

***Example 22: Discovery of Att Site Mutations That Increase the Cloning Efficiency of GATEWAY™ Cloning Reactions***

In experiments designed to understand the determinants of *att* site specificity, point mutations in the core region of *attL* were made. Nucleic acid molecules containing these mutated *attL* sequences were then reacted in an LR

reaction with nucleic acid molecules containing the cognate *attR* site (*i.e.*, an *attR* site containing a mutation corresponding to that in the *attL* site), and recombinational efficiency was determined as described above. Several mutations located in the core region of the att site were noted that either slightly increased (less than 2-fold) or decreased (between 2-4-fold) the efficiency of the recombination reaction (Table 3).

Table 3. *Effects of attL mutations on Recombination Reactions.*

Site	Sequence	Effect on Recombination
attL0	agcctgcttttttataactaagttggcatta	
attL5	agcctgcttttAttataactaagttggcatta	slightly increased
attL6	agcctgcttttttataTtaagttggcatta	slightly increased
attL13	agcctgctttttttatGctaagttggcatta	decreased
attL14	agcctgctttttttatacCaagttggcatta	decreased
attL15	agcctgctttttttatactaGgttggcatta	decreased
consensus	CAACTTnnTnnnAnnAAGTTG	

It was also noted that these mutations presumably reflected changes that either increased or decreased, respectively, the relative affinity of the integrase protein for binding the core att site. A consensus sequence for an integrase core-binding site (CAACTTNNT) has been inferred in the literature but not directly tested (see, *e.g.*, Ross and Landy, *Cell* 33:261-272 (1983)). This consensus core integrase-binding sequence was established by comparing the sequences of each of the four core att sites found in attP and attB as well as the sequences of five non-att sites that resemble the core sequence and to which integrase has been shown to bind in vitro. These experiments suggest that many more att site mutations might be identified which increase the binding of integrase to the core att site and thus increase the efficiency of GATEWAY™ cloning reactions.

**Example 23: Effects of Core Region Mutations on Recombination Efficiency**

To directly compare the cloning efficiency of mutations in the att site core region, single base changes were made in the attB2 site of an attB1-TET-attB2 PCR product. Nucleic acid molecules containing these mutated attB2 sequences were then reacted in a BP reaction with nucleic acid molecules containing non-cognate attP sites (i.e., wildtype attP2), and recombinational efficiency was determined as described above. The cloning efficiency of these mutant attB2 containing PCR products compared to standard attB1-TET-attB2 PCR product are shown in Table 4.

*Table 4. Efficiency of Recombination With Mutated attB2 Sites.*

<u>Site</u>	<u>Sequence</u>	<u>Mutation</u>	<u>Cloning Efficiency</u>
attB0	tcaagttagttataaaaaagcaggct		
attB1	ggggacaagtttgtacaaaaagcaggct		
attB2	ggggaccactttgtacaagaaagctgggt		100%
attB2.1	gggggAacactttgtacaagaaagctgggt	C→A	40%
attB2.2	ggggacAactttgtacaagaaagctgggt	C→A	131%
attB2.3	ggggaccCctttgtacaagaaagctgggt	A→C	4%
attB2.4	ggggaccaAttgtgtacaagaaagctgggt	C→A	11%
attB2.5	ggggaccacGttgtacaagaaagctgggt	T→G	4%
attB2.6	ggggaccactGtgtacaagaaagctgggt	T→G	6%
attB2.7	ggggaccacttGgtacaagaaagctgggt	T→G	1%
attB2.8	ggggaccactttTtacaagaaagctgggt	G→T	0.5%

As noted above, a single base change in the attB2.2 site increased the cloning efficiency of the attB1-TET-attB2.2 PCR product to 131% compared to the attB1-TET-attB2 PCR product. Interestingly, this mutation changes the integrase core binding site of attB2 to a sequence that matches more closely the proposed consensus sequence.

Additional experiments were performed to directly compare the cloning efficiency of an attB1-TET-attB2 PCR product with a PCR product that contained attB sites containing the proposed consensus sequence (*see* Example 22) of an integrase core binding site. The following attB sites were used to amplify attB-TET PCR products:

attB1      ggggacaagtttgtacaaaaaagcaggct  
attB1.6    ggggacaaCtttgtacaaaaaagTTggct  
attB2      ggggaccactttgtacaagaaagctgggt  
attB2.10   ggggacAactttgtacaagaaagTtgggt

BP reactions were carried out between 300 ng (100 fmoles) of pDONR201 (Figure 49A) with 80 ng (80 fmoles) of attB-TET PCR product in a 20  $\mu$ l volume with incubation for 1.5 hrs at 25°C, creating pENTR201-TET Entry clones. A comparison of the cloning efficiencies of the above-noted attB sites in BP reactions is shown in Table 5.

*Table 5. Cloning efficiency of BP Reactions.*

PCR product	CFU/ml	Fold Increase
B1-tet-B2	7,500	
B1.6-tet-B2	12,000	1.6 x
B1-tet-B2.10	20,900	2.8 x
B1.6-tet-B2.10	30,100	4.0 x

These results demonstrate that attB PCR products containing sequences that perfectly match the proposed consensus sequence for integrase core binding sites can produce Entry clones with four-fold higher efficiency than standard Gateway attB1 and attB2 PCR products.

The entry clones produced above were then transferred to pDEST20 (Figure 40A) via LR reactions (300 ng (64 fmoles) pDEST20 mixed with 50 ng (77 fmoles) of the respective pENTR201-TET Entry clone in 20  $\mu$ l volume; incubated for 1 hr incubation at 25°C). The efficiencies of cloning for these reactions are compared in Table 6.

Table 6. Cloning Efficiency of LR Reactions.

pENTR201-TET x pDEST20	CFU/ml	Fold Increase
L1-tet-L2	5,800	
L1.6-tet-L2	8,000	1.4
L1-tet-L2.10	10,000	1.7
L1.6-tet-L2.10	9,300	1.6

These results demonstrate that the mutations introduced into attB1.6 and attB2.10 that transfer with the gene into entry clones slightly increase the efficiency of LR reactions. Thus, the present invention encompasses not only mutations in *attB* sites that increase recombination efficiency, but also to the corresponding mutations that result in the *attL* sites created by the BP reaction.

To examine the increased cloning efficiency of the attB1.6-TET-attB2.10 PCR product over a range of PCR product amounts, experiments analogous to those described above were performed in which the amount of attB PCR product was titrated into the reaction mixture. The results are shown in Table 7.

Table 7. Titration of attB PCR products.

Amount of attB PCR product (ng)	PCR product	CFU/ml	Fold Increase
20	attB1-TET-attB2	3,500	6.1
	attB1.6-TET-attB2.10	21,500	
50	attB1-TET-attB2	9,800	5.0
	attB1.6-TET-attB2.10	49,000	
100	attB1-TET-attB2	18,800	2.8
	attB1.6-TET-attB2.10	53,000	
200	attB1-TET-attB2	19,000	2.5
	attB1.6-TET-attB2.10	48,000	

These results demonstrate that as much as a six-fold increase in cloning efficiency is achieved with the attB1.6-TET-attB2.10 PCR product as compared to the standard attB1-TET-attB2 PCR product at the 20 ng amount.

**Example 24: Determination of attB Sequence Requirements for Optimum Recombination Efficiency**

To examine the sequence requirements for attB and to determine which attB sites would clone with the highest efficiency from populations of degenerate attB sites, a series of experiments was performed. Degenerate PCR primers were designed which contained five bases of degeneracy in the B-arm of the attB site. These degenerate sequences would thus transfer with the gene into Entry clone in BP reactions and subsequently be transferred with the gene into expression clones in LR reactions. The populations of degenerate attB and attL sites could thus be cycled from attB to attL back and forth for any number of cycles. By altering the reaction conditions at each transfer step (for example by decreasing the reaction time and/or decreasing the concentration of DNA) the reaction can be made increasingly more stringent at each cycle and thus enrich for populations of attB and attL sites that react more efficiently.

The following degenerate PCR primers were used to amplify a 500 bp fragment from pUC18 which contained the lacZ alpha fragment (only the attB portion of each primer is shown):

```

attB1      GGGG ACAAGTTTGTACAAA AAAGC AGGCT
attB1n16-20 GGGG ACAAGTTTGTACAAA nnnnn AGGCT
attB1n21-25 GGGG ACAAGTTTGTACAAA AAAGC nnnnn

attB2      GGGG ACCACTTTGTACAAG AAAGC TGGGT
attB2n16-20 GGGG ACCACTTTGTACAAG nnnnn TGGGT
attB2n21-25 GGGG ACCACTTTGTACAAG AAAGC nnnnn

```

The starting population size of degenerate att sites is  $4^5$  or 1024 molecules. Four different populations were transferred through two BP reactions and two LR reactions. Following transformation of each reaction, the population of transformants was amplified by growth in liquid media containing the appropriate selection antibiotic. DNA was prepared from the population of clones by alkaline

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lysis miniprep and used in the next reaction. The results of the BP and LR cloning reactions are shown below.

## BP-1, overnight reactions

	cfu/ml	percent of control
attB1-LacZa-attB2	78,500	100 %
attB1n16-20-LacZa-attB2	1,140	1.5 %
attB1n21-25-LacZa-attB2	11,100	14 %
attB1-LacZa-attB2n16-20	710	0.9 %
attB1-LacZa-attB2n21-25	16,600	21 %

LR-1, pENTR201-LacZa x pDEST20/*EcoRI*, 1hr reactions

	cfu/ml	percent of control
attL1-LacZa-attL2	20,000	100 %
attL1n16-20-LacZa-attL2	2,125	11 %
attL1n21-25-LacZa-attL2	2,920	15 %
attL1-LacZa-attL2n16-20	3,190	16 %
attL1-LacZa-attL2n21-25	1,405	7 %

BP-2, pEXP20-LacZa/*ScaI* x pDONR 201, 1hr reactions

	cfu/ml	percent of control
attB1-LacZa-attB2	48,600	100 %
attB1n16-20-LacZa-attB2	22,800	47 %
attB1n21-25-LacZa-attB2	31,500	65 %
attB1-LacZa-attB2n16-20	42,400	87 %
attB1-LacZa-attB2n21-25	34,500	71 %

LR-2, pENTR201-LacZa x pDEST6/*NcoI*, 1hr reactions

	cfu/ml	percent of control
attL1-LacZa-attL2	23,000	100 %
attL1n16-20-LacZa-attL2	49,000	213 %
attL1n21-25-LacZa-attL2	18,000	80 %
attL1-LacZa-attL2n16-20	37,000	160 %
attL1-LacZa-attL2n21-25	57,000	250 %

These results demonstrate that at each successive transfer, the cloning efficiency of the entire population of att sites increases, and that there is a great deal of flexibility in the definition of an attB site. Specific clones may be isolated from the above reactions, tested individually for recombination efficiency, and

sequenced. Such new specificities may then be compared to known examples to guide the design of new sequences with new recombination specificities. In addition, based on the enrichment and screening protocols described herein, one of ordinary skill can easily identify and use sequences in other recombination sites, *e.g.*, other *att* sites, *lox*, FRT, etc., that result in increased specificity in the recombination reactions using nucleic acid molecules containing such sequences.

***Example 25: Design of att Site PCR Adapter-Primers***

Additional studies were performed to design gene-specific primers with 12bp of attB1 and attB2 at their 5'-ends. The optimal primer design for *att*-containing primers is the same as for any PCR primers: the gene-specific portion of the primers should ideally have a  $T_m$  of  $> 50^\circ\text{C}$  at 50 mM salt (calculation of  $T_m$  is based on the formula  $59.9 + 41(\%GC) - 675/n$ ).

**Primers:**

12bp attB1: AA AAA GCA GGC TNN - forward gene-specific primer

12bp attB2: A GAA AGC TGG GTN - reverse gene-specific primer

attB1 adapter primer: GGGGACAAGTTTGTACAAAAAAGCAGGCT

attB2 adapter primer: GGGGACCACTTTGTACAAGAAAGCTGGGT

**Protocol:**

(1) Mix 200 ng of cDNA library or 1 ng of plasmid clone DNA (alternatively, genomic DNA or RNA could be used) with 10 pmoles of gene specific primers in a 50  $\mu\text{l}$  PCR reaction, using one or more polypeptides having DNA polymerase activity such as those described herein. (The addition of greater than 10 pmoles of gene-specific primers can decrease the yield of attB PCR product. In addition, if RNA is used, a standard reverse transcriptase-PCR (RT-



PCR) protocol should be followed; *see, e.g.,* Gerard, G.F., *et al.*, *FOCUS* 11:60 (1989); Myers, T.W., and Gelfand, D.H., *Biochem.* 30:7661 (1991); Freeman, W.N., *et al.*, *BioTechniques* 20:782 (1996); and U.S. Application No. 09/064,057, filed April 22, 1998, the disclosures of all of which are incorporated herein by reference.)

1<sup>st</sup> PCR profile:

(a) 95°C for 3 minutes

(b) 10 cycles of:

(i) 94°C for 15 seconds

(ii) 50°C\* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(c) 68°C for 5 minutes

(d) 10°C hold

\*The optimal annealing temperature is determined by the calculated T<sub>m</sub> of the gene-specific part of the primer.

(2) Transfer 10 µl to a 40 µl PCR reaction mix containing 35 pmoles each of the attB1 and attB2 adapter primers.

2<sup>nd</sup> PCR profile:

(a) 95°C for 1 minute

(b) 5 cycles of:

(i) 94°C for 15 seconds

(ii) 45°C\* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(c) 15-20 cycles\*\* of:

(i) 94°C for 15 seconds

(ii) 55°C\* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(d) 68°C for 5 minutes

(e) 10°C hold

5        \*The optimal annealing temperature is determined by the calculated  $T_m$  of the gene-specific part of the primer.

\*\*15 cycles is sufficient for low complexity targets.

Notes:

- 10        1. It is useful to perform a no-adapter primer control to assess the yield of attB PCR product produced.
2. Linearized template usually results in slightly greater yield of PCR product.

15        ***Example 26: One-Tube Recombinational Cloning Using the GATEWAY™ Cloning System***

20        To provide for easier and more rapid cloning using the GATEWAY™ cloning system, we have designed a protocol whereby the BP and LR reactions may be performed in a single tube (a "one-tube" protocol). The following is an example of such a one-tube protocol; in this example, an aliquot of the BP reaction is taken before adding the LR components, but the BP and LR reactions may be performed in a one-tube protocol without first taking the BP aliquot:

25

<u>Reaction Component</u>	<u>Volume</u>
attB DNA (100-200 ng/25 µl reaction)	1-12.5 µl
attP DNA (pDONR201) 150 ng/µl	2.5 µl
5X BP Reaction Buffer	5.0 µl
30        Tris-EDTA	(to 20 µl)
<u>BP Clonase</u>	<u>5.0 µl</u>
Total vol.	25 µl

After the above components were mixed in a single tube, the reaction mixtures were incubated for 4 hours at 25°C. A 5 µl aliquot of reaction mixture was removed, and 0.5 µl of 10X stop solution was added to this reaction mixture and incubated for 10 minutes at 37°C. Competent cells were then transformed with 1-2 µl of the BP reaction per 100 µl of cells; this transformation yielded colonies of Entry Clones for isolation of individual Entry Clones and for quantitation of the BP Reaction efficiency.

To the remaining 20 µl of BP reaction mixture, the following components of the LR reaction were added:

<u>Reaction Component</u>	<u>Final Concentration</u>	<u>Volume Added</u>
NaCl	0.75 M	1 µl
Destination Vector	150 ng/ul	3 µl
<u>LR Clonase</u>		<u>6 µl</u>
Total vol.		30 µl

After the above components were mixed in a single tube, the reaction mixtures were incubated for 2 hours at 25°C. 3 µl of 10X stop solution was added, and the mixture was incubated for 10 minutes at 37°C. Competent cells were then transformed with 1-2 µl of the reaction mixture per 100 µl of cells

Notes:

1. If desired, the Destination Vector can be added to the initial BP reaction.
2. The reactions can be scaled down by 2x, if desired.
3. Shorter incubation times for the BP and/or LR reactions can be used (scaled to the desired cloning efficiencies of the reaction), but a lower number of colonies will typically result.
4. To increase the number of colonies obtained by several fold, incubate the BP reaction for 6-20 hours and increase the LR reaction to 3 hours. Electroporation also works well with 1-2 ul of the PK-treated reaction mixture.

5. PCR products greater than about 5 kb may show significantly lower cloning efficiency in the BP reaction. In this case, we recommend using a one-tube reaction with longer incubation times (*e.g.*, 6-18 hours) for both the BP and LR steps.

***Example 27: Relaxation of Destination Vectors During the LR Reaction***

To further optimize the LR Reaction, the composition of the LR Reaction buffer was modified from that described above and this modified buffer was used in a protocol to examine the impact of enzymatic relaxation of Destination Vectors during the LR Reaction.

LR Reactions were set up as usual (*see, e.g.*, Example 6), except that 5X BP Reaction Buffer (*see* Example 5) was used for the LR Reaction. To accomplish Destination Vector relaxation during the LR Reaction, Topoisomerase I (Life Technologies, Inc., Rockville, MD; Catalogue No. 38042-016) was added to the reaction mixture at a final concentration of ~15U per  $\mu\text{g}$  of total DNA in the reaction (for example, for reaction mixtures with a total of 400ng DNA in the 20  $\mu\text{l}$  LR Reaction, ~6units of Topoisomerase I was added). Reaction mixtures were set up as follows:

<u>Reaction Component</u>	<u>Volume</u>
ddH <sub>2</sub> O	6.5 $\mu\text{l}$
4X BP Reaction Buffer	5 $\mu\text{l}$
100ng single chain/linear pENTR CAT, 50 ng/ $\mu\text{l}$	2 $\mu\text{l}$
300ng single chain/linear pDEST6, 150ng/ $\mu\text{l}$	2 $\mu\text{l}$
Topoisomerase I, 15 U/ml	0.5 $\mu\text{l}$
LR Clonase	4 $\mu\text{l}$

Reaction mixtures were incubated at 25°C for 1hour, and 2  $\mu\text{l}$  of 2  $\mu\text{g}/\mu\text{l}$  Proteinase K was then added and mixtures incubated for 10 minutes at 37°C to stop the LR Reaction. Competent cells were then transformed as described in the preceding examples. The results of these studies demonstrated that relaxation of

substrates in the LR reaction using Topoisomerase I resulted in a 2- to 10-fold increase in colony output compared to those LR reactions performed without including Topoisomerase I.

5           Having now fully described the present invention in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or  
10 any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

          All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent  
15 as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

167.1

Applicant's or agent's file reference number	0942-J8PC03	International application No. <sup>tl</sup>	PCT/US 00/05432
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**  
(PCT Rule 13bis)

REC'D 17 APR 2000

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A. The indications made below relate to the microorganism referred to in the description on page 52, line 31.

**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

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Agricultural Research Culture Collection (NRRL)  
International Depositary Authority

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1815 N. University Street  
Peoria, Illinois 61604  
United States of America

Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30099

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pAHPKan) or Escherichia coli DB3.1(pAttPKan)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

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Applicant's or agent's file reference number	0942.468PC03	International application No. tl <b>PCT/US 00/05432</b>
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A. The indications made below relate to the microorganism referred to in the description on page 35, line 16.

**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

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International Depositary Authority

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Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30100

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pENTR-1A)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

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Barbara Fridis

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167 3

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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
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(PCT Rule 13bis)

REC 1 1999

A. The indications made below relate to the microorganism referred to in the description on page 16, line 33

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**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

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Date of deposit  
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**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pENTR-2B)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

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P. G. F. F. F.

P. G. F. F. F. 3230 (F)

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167.4

Applicant's or agent's file reference number	0942.468PC03	International Application No. <b>PCT/US 0/05432</b>
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
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A. The indications made below relate to the microorganism referred to in the description on page 55, line 16.

**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

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February 27, 1999

Accession Number  
NRRL B-30102

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pENTR-3C)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

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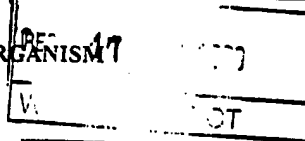
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167.5

Applicant's or agent's file reference number	0942.468PC03	International application No. tb PCT/US 00/05432
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
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(PCT Rule 13bis)



A. The indications made below relate to the microorganism referred to in the description on 8	REC'D 17 APR 2000 WIPO PCT
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**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

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Accession Number  
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**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pEZC15101)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

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167.6

Applicant's or agent's file reference number	0942.468PC03	International application No. 1.	PCT/US 00/05432
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**  
(PCT Rule 13bis)

REC'D 17

WIPO

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30104
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pEZC15102)	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
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167.7

Applicant's or agent's file reference number	0942.468PC03	International application No. <b>PCT/US</b>	<b>00/05432</b>
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INDICATIONS RELATING TO DEPOSITED MICROORGANISMS  
OR OTHER BIOLOGICAL MATERIAL  
(PCT Rule 13bis)

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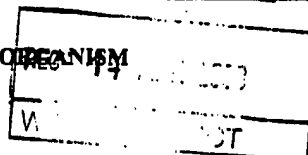
A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
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Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30105
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB3.1(pEZC15103)	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
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167.8

Applicant's or agent's file reference number	0942.408PC03	International application No. tl <b>PCT/US 00/05432</b>
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**  
(PCT Rule 13bis)



A. The indications made below relate to the microorganism referred to in the description on page 51, line 20-21.

**B. IDENTIFICATION OF DEPOSIT**

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International Depository Authority

Address of depositary institution (including postal code and country)

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United States of America

Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30108

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)

This information is continued on an additional sheet ☐

Escherichia coli DB10B(pCMVSPORT6)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)

**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

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## WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group of nucleotide sequences consisting of an attB1 nucleotide sequence as set forth in Figure 9, an attB2 nucleotide sequence as set forth in Figure 9, an attP1 nucleotide sequence as set forth in Figure 9, an attP2 nucleotide sequence as set forth in Figure 9, an attL1 nucleotide sequence as set forth in Figure 9, an attL2 nucleotide sequence as set forth in Figure 9, an attR1 nucleotide sequence as set forth in Figure 9, an attR2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, and a mutant, fragment, or derivative thereof.

2. An isolated nucleic acid molecule comprising an attB1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

3. An isolated nucleic acid molecule comprising an attB2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

4. An isolated nucleic acid molecule comprising an attP1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

5. An isolated nucleic acid molecule comprising an attP2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

6. An isolated nucleic acid molecule comprising an attL1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

7. An isolated nucleic acid molecule comprising an attL2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

5 8. An isolated nucleic acid molecule comprising an attR1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

10 9. An isolated nucleic acid molecule comprising an attR2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

15 10. The isolated nucleic acid molecule of claim 1, further comprising one or more functional or structural nucleotide sequences selected from the group consisting of one or more multiple cloning sites, one or more localization signals, one or more transcription termination sites, one or more transcriptional regulatory sequences, one or more translational signals, one or more origins of replication, one or more fusion partner peptide-encoding nucleic acid molecules, one or more protease cleavage sites, and one or more 5' polynucleotide extensions.

20 11. The nucleic acid molecule of claim 10, wherein said transcriptional regulatory sequence is a promoter, an enhancer, or a repressor.

25 12. The nucleic acid molecule of claim 10, wherein said fusion partner peptide-encoding nucleic acid molecule encodes glutathione S-transferase (GST), hexahistidine (His<sub>6</sub>), or thioredoxin (Trx).

30 13. The nucleic acid molecule of claim 10, wherein said 5' polynucleotide extension consists of from one to five nucleotide bases.

14. The nucleic acid molecule of claim 13, wherein said 5' polynucleotide extension consists of four or five guanine nucleotide bases.

15. A primer nucleic acid molecule suitable for amplifying a target nucleotide sequence, comprising the isolated nucleic acid molecule of claim 1 or a portion thereof linked to a target-specific nucleotide sequence useful in amplifying said target nucleotide sequence.

16. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB1 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

17. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB2 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

18. The primer nucleic acid molecule of claim 15, further comprising a 5' terminal extension of four or five guanine bases.

19. A vector comprising the isolated nucleic acid molecule of claim 1.

20. The vector of claim 19, wherein said vector is an Expression Vector.

21. A host cell comprising the isolated nucleic acid molecule of claim 1 or the vector of claim 19.

22. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said



templates and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said second primer is homologous to or complementary to at least a portion of said first primer; and

- 5 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one or both termini of said molecules.

10 23. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- 15 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said templates and at least a portion of a recombination site, and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said recombination site on said second primer is complementary to or homologous to at least a portion of said recombination site on said first primer; and
- 20 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one or both termini of said molecules.
- 25

30 24. A method of amplifying or synthesizing one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity

and one or more first primers comprising at least a portion of a recombination site and a template-specific sequence that is complementary to or capable of hybridizing to said template;

(b) incubating said mixture under conditions sufficient to synthesize or amplify one or more first nucleic acid molecules complementary to all or a portion of said templates wherein said molecules comprise at least a portion of a recombination site at one or both termini of said molecules;

(c) mixing said molecules with one or more second primers comprising one or more recombination sites, wherein said recombination sites of said second primers are homologous to or complementary to at least a portion of said recombination sites on said first nucleic acid molecules; and

(d) incubating said mixture under conditions sufficient to synthesize or amplify one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules and which comprise one or more recombination sites at one or both termini of said molecules.

25. A polypeptide encoded by the isolated nucleic acid molecule of any one of claims 1-10.

26. An isolated nucleic acid molecule comprising one or more *att* recombination sites comprising at least one mutation in its core region that increases the specificity of interaction between said recombination site and a second *att* recombination site.

27. The isolated nucleic acid molecule of claim 26, wherein said mutation is at least one substitution mutation of at least one nucleotide in the seven basepair overlap region of said core region of said recombination site.

28. The isolated nucleic acid molecule of claim 26, wherein said nucleic acid molecule comprises the sequence NNNATAC, wherein "N" refers to any nucleotide with the proviso that if one of the first three nucleotides in the consensus sequence is a T/U, then at least one of the other two of the first three nucleotides is not a T/U.

29. An isolated nucleic acid molecule comprising one or more mutated *att* recombination sites comprising at least one mutation in its core region that enhances the efficiency of recombination between a first nucleic acid molecule comprising said mutated *att* recombination site and a second nucleic acid molecule comprising a second recombination site that interacts with said mutated *att* recombination site.

30. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site is a mutated *attL* site comprising a core region having the nucleotide sequence caactnntnnnannaagttg, wherein "n" represents any nucleotide.

31. The isolated nucleic acid molecule of claim 30, wherein said mutated *attL* recombination site comprises a core region having a nucleotide sequence selected from agcctgctttattataactaagttggcatta (*attL5*) and agcctgctttttatattaagttggcatta (*attL6*).

32. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site comprises a core region having a nucleotide sequence selected from the group consisting of ggggacaactttgtacaaaaagttggct (*attB1.6*), ggggacaactttgtacaagaagctgggt (*attB2.2*), and ggggacaactttgtacaagaagttgggt (*attB2.10*).

33. A vector selected from the group consisting of pENTR1A, pENTR2B, pENTR3C, pENTR4, pENTR5, pENTR6, pENTR7, pENTR8, pENTR9, pENTR10, pENTR11, pDEST1, pDEST2, pDEST3, pDEST4,

pDEST5, pDEST6, pDEST7, pDEST8, pDEST9, pDEST10, pDEST11, pDEST12.2 (also known as pDEST12), pDEST13, pDEST14, pDEST15, pDEST16, pDEST17, pDEST18, pDEST19, pDEST20, pDEST21, pDEST22, pDEST23, pDEST24, pDEST25, pDEST26, pDEST27, pDEST28, pDEST29, pDEST30, pDEST31, pDEST32, pDEST33, pDEST34, pDONR201 (also known as pENTR21 attP vector or pAttPkan Donor Vector), pDONR202, pDONR203 (also known as pEZ15812), pDONR204, pDONR205, pDONR206 (also known as pENTR22 attP vector or pAttPgen Donor Vector), pDONR207, pMAB58, pMAB62, pMAB85 and pMAB86.

34. A host cell comprising the vector of claim 33.

35. A polypeptide encoded by the vector of claim 33.

36. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the isolated nucleic acid molecule of any one of claims 1-10, 26 and 29.

37. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the primer of claim 15 or claim 18.

38. A kit for use in cloning a nucleic acid molecule, said kit comprising the vector of claim 19 or claim 33.

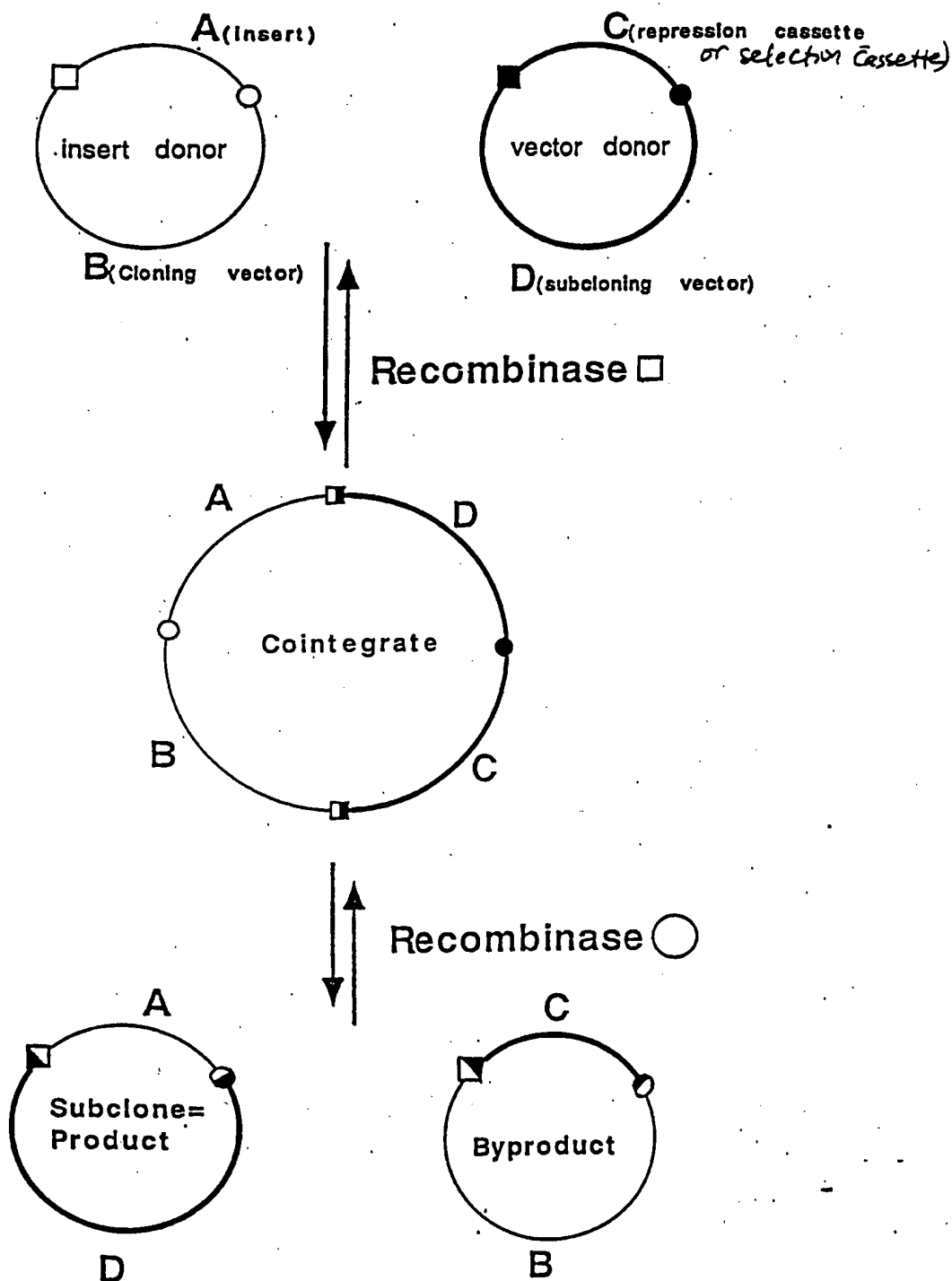


Figure 1

2/240

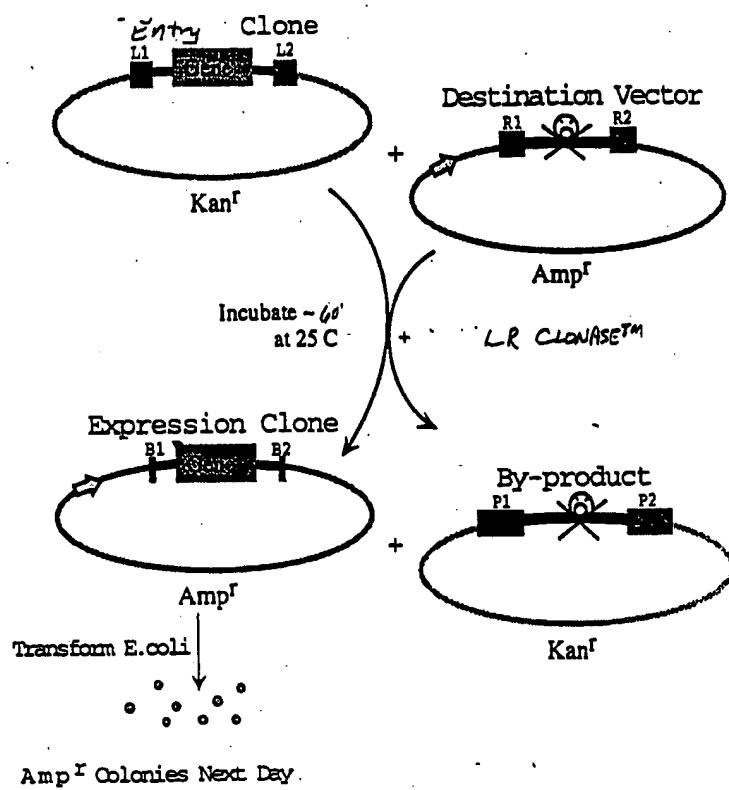


FIGURE 2

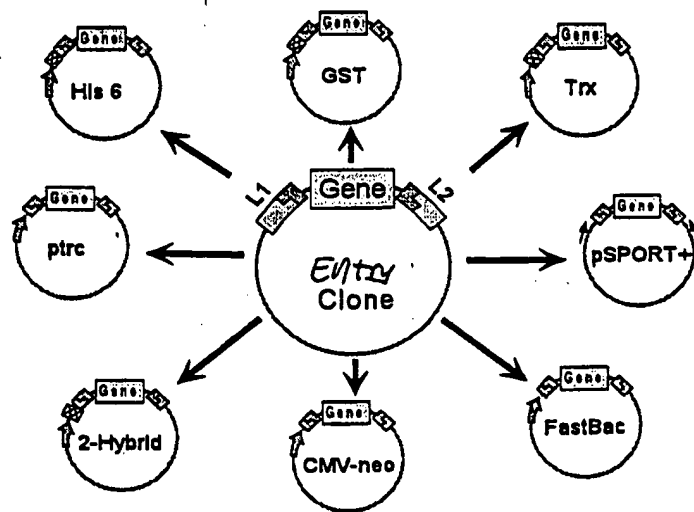


FIGURE 3

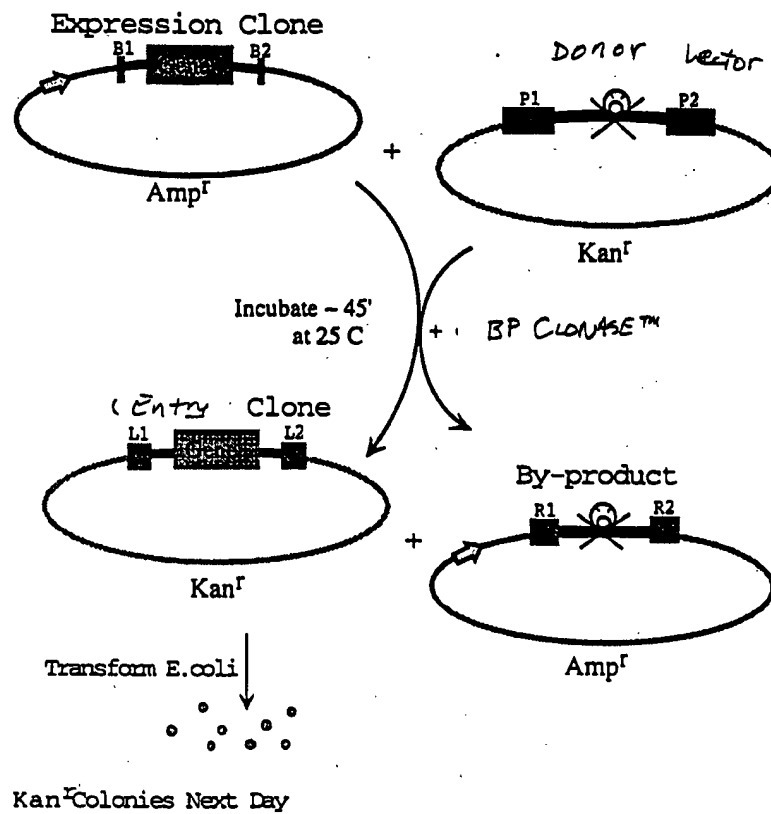


FIGURE 4



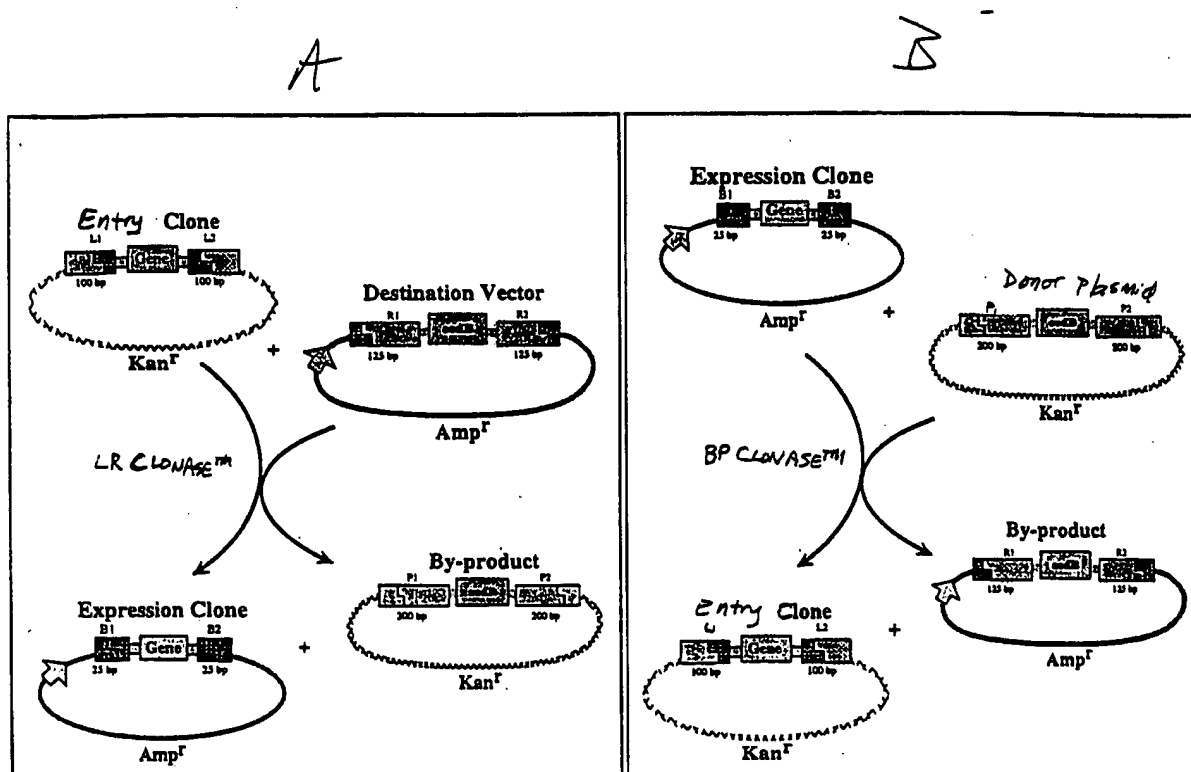


FIGURE 5

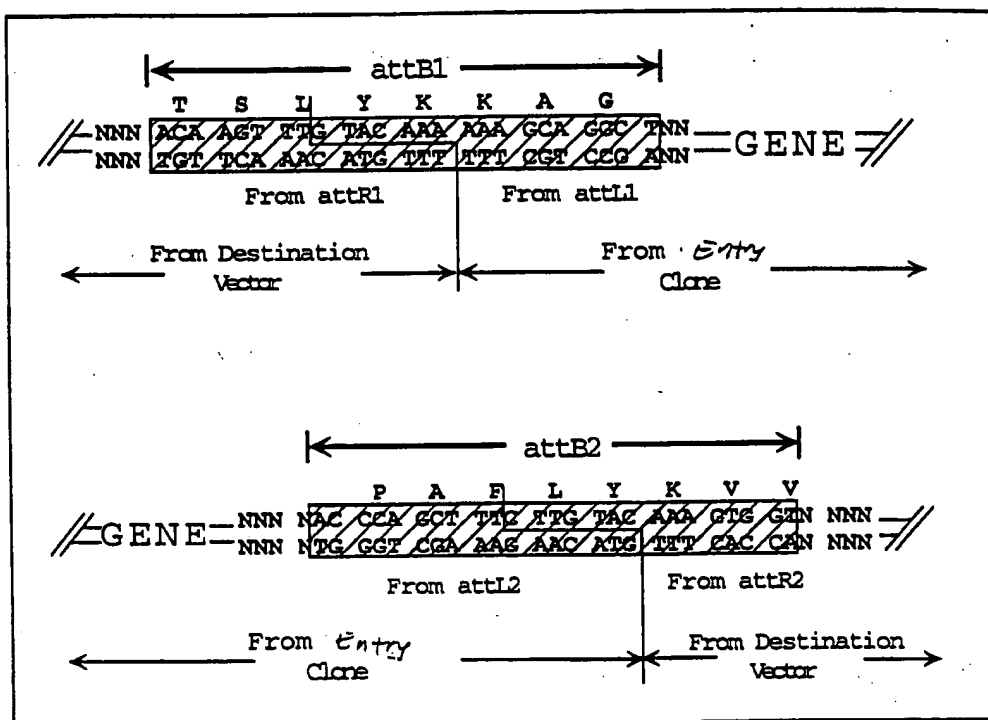


FIGURE 6

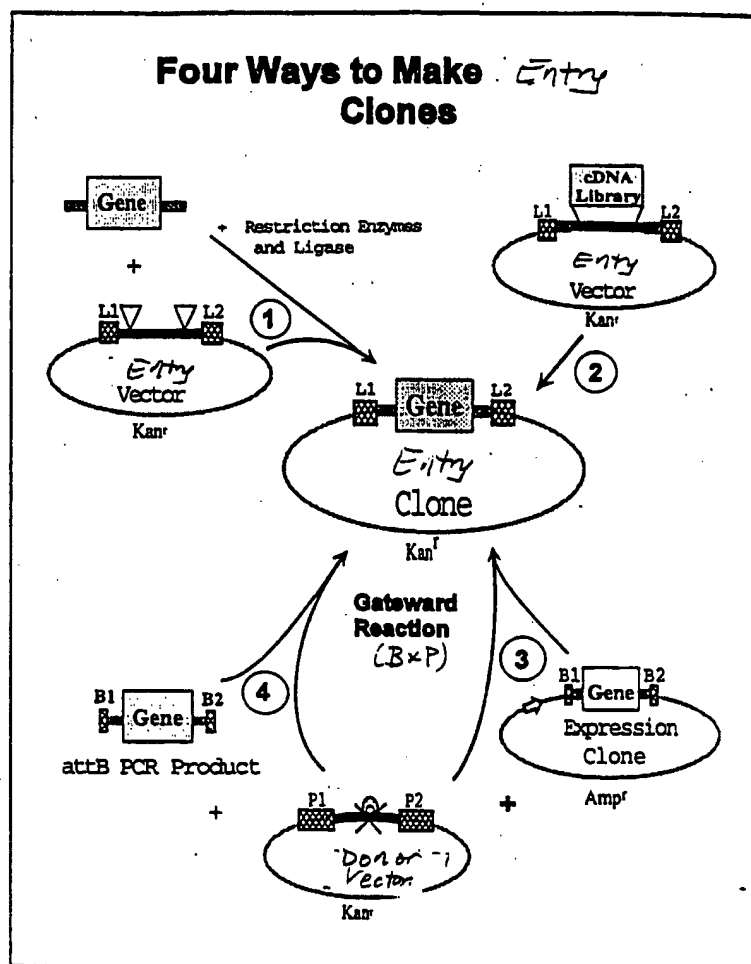


FIGURE 7

8/240

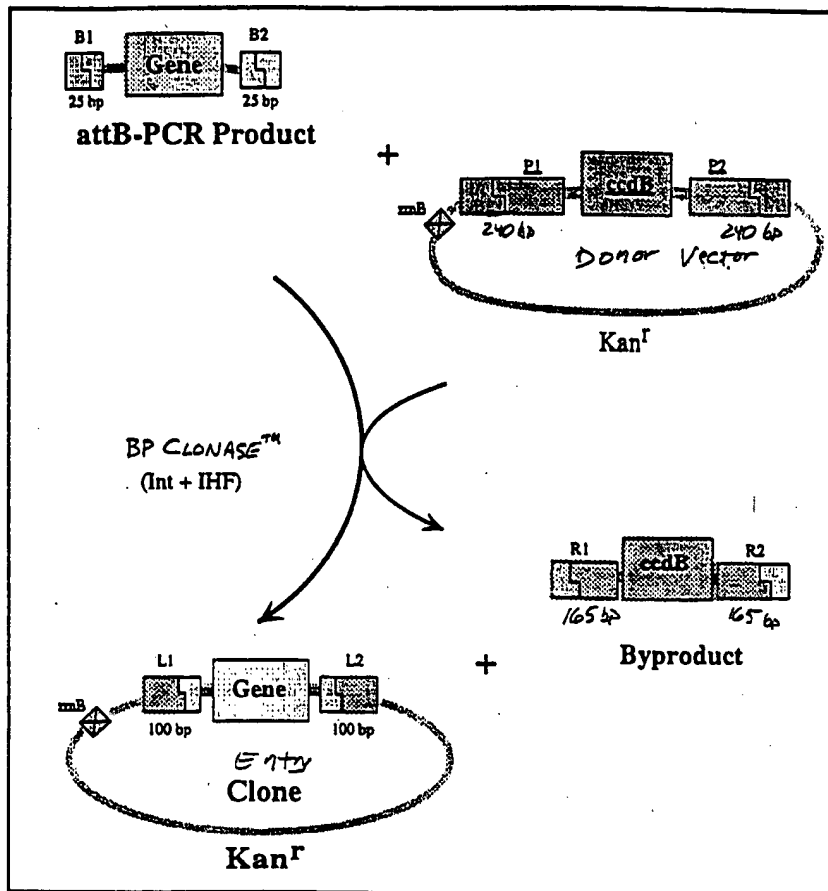


FIGURE 8

### Recombination Site Nucleotide Sequences

attB1: 5'-ACAAGTTTGTACAAAAAAGCAGGCT-3'

attB2: 5'-ACCCAGCTTTCTTGTACAAAGTGGT-3'

attP1: 5'-TACAGGTCCTAATACCATCTAAGTAGTTGATTCATAGTGACTGGATATG-  
TTGTGTTTTACAGTATTATGTAGTCTGTTTTTATGCAAAATCTAATTTA-  
ATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTTTTTTGTAC-  
AAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACA-  
GGTCACTATCAGTCAAAATAAAATCATTATTTG-3'

attP2: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACAAAT-  
TGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTGAAC-  
GAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCAT-  
AAAAACAGACTACATAATACTGTAAACACAACATATCCAGTCACTATGA-  
ATCAACTACTTAGATGGTATTAGTGACCTGTA-3'

attR1: 5'-ACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAA-  
TATCAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATAC-  
TGTAACACAACATATCCAGTCACTATG-3'

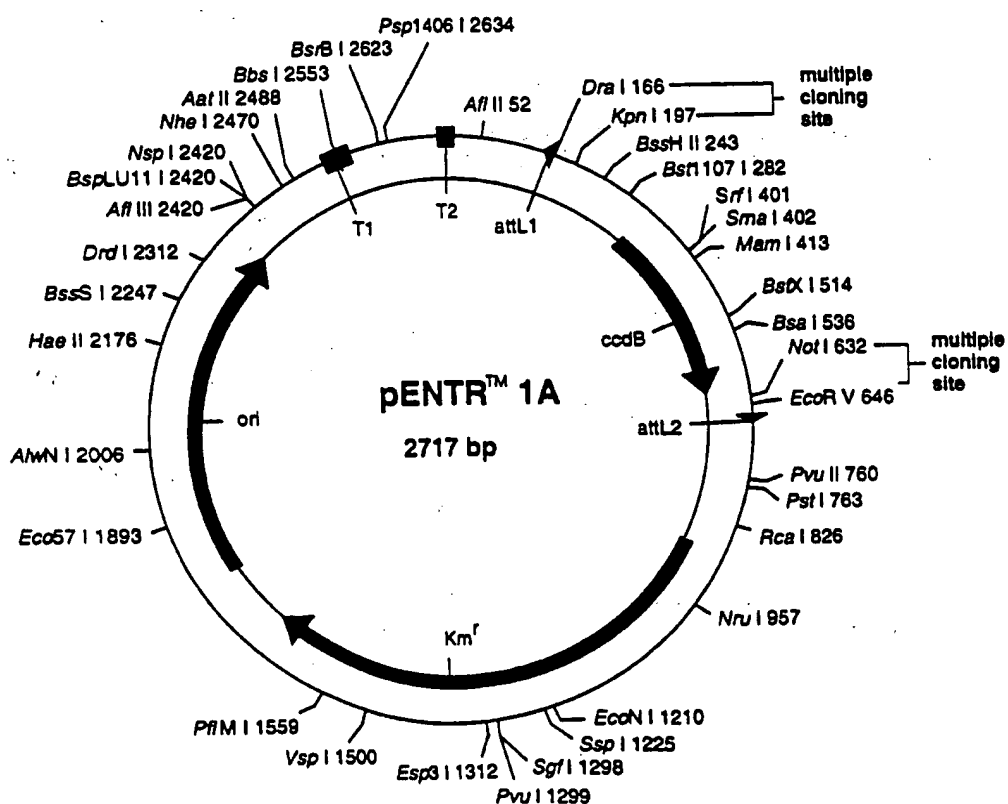
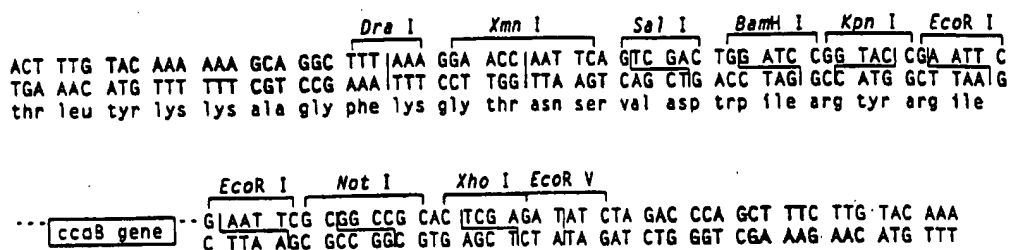
attR2: 5'-GCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTAT-  
GTAGTCTGTTTTTATGCAAAATCTAATTTAATATATTGATATTT-  
ATATCATTTTACGTTTCTCGTTCAGCTTTCTTGTACAAAGTGGT-3'

attL1: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAAC-  
AAATTGATAAGCAATGCTTTTTTATAATGCCAACTTTGTACAAAAAA-  
GCAGGCT-3'

attL2: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACAA-  
ATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTGGGT-3'

Figure 9

**Figure 10A: Cloning sites of the Entry Vector pENTR1A (reading frame A)**



## pENTR1A 2717 bp

<u>Base Nos.</u>	<u>Gene Encoded</u>
67..166	attL1
321..626	ccdB
655..754	attL2
877..1686	KmR
1791..2364	ori

```

1 CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTTTAA AGGAACCAAT
181 TCAGTCGACT GGATCCGGTA CCGAATTCGC TTAATAAAG CCAGATAACA GTATGCGTAT
241 TTGCGCGCTG ATTTTTCGGG TATAAGAATA TATACTGATA TGTATACCCG AAGTATGTCA
301 AAAAGAGGTG TGCTTCTAGA ATGCAGTTTA AGGTTTACAC CTATAAAAGA GAGAGCCGTT
361 ATCGTCTGTT TGTGGATGTA CAGAGTGATA TTATTGACAC GCCCGGGCGA CGGATAGTGA
421 TCCCCCTGGC CAGTGACCGT CTGCTGTCAG ATAAAGTCTC CCGTGAACCT TACCCGGTGG
481 TGTGATTCGG GGATGAAAGC TGGCGCATGA TGACCACCGA TATGGCCAGT GTGCCGCTCT
541 CCGTTATCGG GGAAGAAGTG GCTGATCTCA GCCACCGCGA AAATGACATC AAAAACGCCA
601 TTAACCTGAT GTTCTGGGGA ATATAGAATT CGCGGCCGCA CTCGAGATAT CTAGACCCAG
661 CTTTCTTGTA CAAAGTTGGC ATTATAAGAA AGCATTGCTT ATCAATTTGT TGCAACGAAC
721 AGGTCACTAT CAGTCAAAAT AAAATCATT TTTGCCATCC AGCTGCAGCT CTGGCCCGTG
781 TCTCAAAATC TCTGATGTTA CATTGCACAA GATAAAAATA TATCATCATG AACAAATAAA
841 CTGTCTGCTT ACATAAACAG TAATACAAGG GGTGTTATGA GCCATATTCA ACGGGAAACG
901 TCGAGGCCGC GATTAAATTC CAACATGGAT GCTGATTAT ATGGGTATAA ATGGGGTCGC
961 GATAATGTCG GGCAATCAGG TCGGACAATC TATCGCTTGT ATGGGAAGCC CGATGCGCCA
1021 GAGTTGTTTT TGAAACATGG CAAAGGTAGC GTTGCCAATG ATGTTACAGA TGAGATGGTC
1081 AGACTAAACT GGCTGACGGA ATTTATGCCT CTTCCGACCA TCAAGCATT TATCCGTAAT
1141 CCTGATGATG CATGGTTACT CACCACTGCG ATCCCCGGAA AAACAGCATT CCAGGTATTA
1201 GAAGAATATC CTGATTCAGG TGAAAATATT GTTGATGCGC TGGCAGTGTC CCTGCGCCGG
1261 TTGCATTGCA TTCCTGTTTG TAATTGTCCT TTTAACAGCG ATCGCGTATT TCGTCTCGCT
1321 CAGGCGCAAT CACGAATGAA TAACGGTTTG GTTGATGCGA GTGATTTTGA TGACGAGCGT
1381 AATGGCTGGC CTGTTGAACA AGTCTGAAA GAAATGCATA AACTTTTGCC ATTCTCACCG
1441 GATTCACTCG TCACTCATGG TGATTTCTCA CTTGATAACC TTATTTTGA CGAGGGGAAA
1501 TTAATAGGTT GTATTGATGT TGGACGAGTC GGAATCGCAG ACCGATACCA GGATCTTGCC
1561 ATCCTATGGA ACTGCCTCGG TGAGTTTCT CCTTCATTAC AGAAACGGCT TTTTCAAAAA
1621 TATGGTATTG ATAATCCTGA TATGAATAAA TTGCAGTTTC ATTTGATGCT CGATGAGTTT
1681 TTCTAATCAG AATTGGTTAA TTGGTTGTAA CATTATTCAG ATTGGGCCCC GTTCCACTGA
1741 GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA
1801 ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG TGGTTTGTGT GCCGGATCAA
1861 GAGCTACCAA CTCTTTTTCG GAAGGTAAC TGGCTCAGCA GAGCGCAGAT ACCAAATACT
1921 GTTCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA ACTCTGTAGC ACCGCCTACA
1981 TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA GTGGCGATAA GTCGTGTCTT
2041 ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC AGCGGTCGGG CTGAACGGGG
2101 GGTTCGTGCA CACAGCCAG CTTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG
2161 CGTGAGCTAT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA AGGCGGACAG GTATCCGGTA
2221 AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC CAGGGGGAAA CGCCTGTGAT
2281 CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGACTTGAGC GTCGATTTTT GTGATGCTCG
2341 TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG CCTTTTACG GTTCTTGGCC
2401 TTTTGCTGGC CTTTGTCTCA CATGTTCTTT CTGCGTTAT CCCCTGATT TGTGGATAAC
2461 CGTATTACCG CTAGCATGGA TCTCGGGGAC GTCTAACTAC TAAGCGAGAG TAGGGAACTG
2521 CCAGGCATCA AATAAACCGA AAGGCTCAGT CGGAAGACTG GGCCTTTCGT TTTATCTGTT
2581 GTTTGTCGGT GAACGCTCTC CTGAGTAGGA CAAATCCGCC GGGAGCGGAT TTGAACGTTG
2641 TGAAGCAACG GCGCGGAGGG TGCGGGGCG GACGCGCGCC ATAAACTGCC AGGCATCAAA
2701 CTAAGCAGAA GGCCATC

```

FIGURE 10B

**Figure 11A: Cloning Sites of the Entry Vector pENTR2B (reading frame B)**

Int	attL1	EheI	XmnI	Sall	BamHI											
TTG	TAC	AAA	AAA	GCA	GGC	TGG	CGC	CGG	AAC	CAA	TTC	AGT	CGA	CTG	GAT	CCG
AAC	ATG	TTT	TTT	CGT	CCG	ACC	GCG	GCC	TTG	GTT	AAG	TCA	GCT	GAC	CTA	GSC
Leu	Tyr	Lys	Lys	Ala	Gly	Trp	Arg	Arg	Asn	Gln	Phe	Ser	Arg	Leu	Asp	Pro

KpnI	EcoRI	EcoRI	NotI	XhoI	EcoRV	XbaI										
GTA	CCG	AAT	TC	ccdB	--G	AAT	TCG	CGG	CCG	CAC	TCG	AGA	TAT	CTA	GAC	CCA
CAT	GGC	TTA	AG			C	TTA	AGC	GCC	GGC	GTG	AGC	TCT	ATA	GAT	CTG
Val	Pro	Asn				Asn	Ser	Arg	Pro	His	Ser	Arg	Tyr	Leu	Asp	Pro

Int	attL2				
GCT	TTC	TTG	TAC	AAA	G
CGA	AAG	AAC	ATG	TTT	C
Ala	Phe	Leu	Tyr	Lys	



## pENTR2B 2718 bp.

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
67..166	attL1
322..627	ccdB
656..755	attL2
878..1687	KmR
1792..2365	ori

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1 CTGACGGATG GCCTTTTTGC GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTGGCG CCGGAACCAA
181 TTCAGTCGAC TGGATCCGGT ACCGAATTCTG CTTACTAAAA GCCAGATAAC AGTATGCGTA
241 TTTGCGCGCT GATTTTTGCG GTATAAGAAT ATATACTGAT ATGTATACCC GAAGTATGTC
301 AAAAAGAGGT GTGCTTCTAG AATGCAGTTT AAGGTTTACA CCTATAAAAG AGAGAGCCGT
361 TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA CGCCCGGGCG ACGGATGGTG
421 ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT CCCGTGAACT TTACCCGGTG
481 GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG ATATGGCCAG TGTGCCGGTC
541 TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG AAAATGACAT CAAAAACGCC
601 ATTAACCTGA TGTCTGGGG AATATAGAAT TCGCGGCCGC ACTCGAGATA TCTAGACCCA
661 GCTTTCTTGT ACAAAGTTGG CATTATAAGA AAGCATTGCT TATCAATTG TTGCAACGAA
721 CAGGTCACCTA TCAGTCAAAA TAAATCATT ATTTGCCATC CAGCTGCAGC TCTGGCCCGT
781 GTCTCAAAAT CTCTGATGTT ACATTGCACA AGATAAAAAT ATATCATCAT GAACAATAAA
841 ACTGCTGCTG TACATAAACA GTAATACAAG GGGTGTTATG AGCCATATTC AACGGGAAC
901 GTCGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA TATGGGTATA AATGGGCTCG
961 CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGCTTG TATGGGAAGC CCGATGCGCC
1021 AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT GATGTTACAG ATGAGATGGT
1081 CAGACTAAAC TGGCTGACGG AATTTATGCC TCTTCCGACC ATCAAGCATT TTATCCGTAC
1141 TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCCGGA AAAACAGCAT TCCAGGTATT
1201 AGAAGAATAT CCTGATTCAG GTGAAAATAT TGTGATGCG CTGGCAGTGT TCCTGCGCCG
1261 GTTGCAATCG ATTCTGTTT GTAATTGTCC TTTAACAGC GATCGCGTAT TTCGTCTCGC
1321 TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTGATGCG AGTGATTTTG ATGACGAGCG
1381 TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT AAACTTTTCG CATCTCCACC
1441 GGATTCAGTC GTCACTCATG GTGATTTCTC ACTTGATAAC CTTATTTTTCG ACGAGGGGAA
1501 ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA GACCGATAAC AGGATCTTGC
1561 CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA CAGAAACGGC TTTTTCAAAA
1621 ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT CATTTGATGC TCGATGAGTT
1681 TTTCTAATCA GAATTGGTTA ATTGGTTGTA ACATTATTCA GATTGGGCCC CGTTCCACTG
1741 AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA GATCCTTTT TTCTGCGCGT
1801 AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG GTGGTTTGTG TGCCGGATCA
1861 AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC
1921 TGTTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC
1981 ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT
2041 TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG
2101 GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA
2161 GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT
2221 AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA
2281 TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC
2341 GTCAGGGGGG CGGAGCCTAT GGAACAAACG CAGCAACGCG GCCTTTTTAC GGTTCCTGGC
2401 CTTTTGCTCG CTTTTGCTC ACATGTTCTT TCCTGCGTTA TCCCTGATT CTGTGATAA
2461 CCGTATTACC GCTAGCATGG ATCTCGGGGA CGTCTAACTA CTAAGCGAGA GTAGGGAAC
2521 GCCAGGCATC AAATAAACG AAAGGCTCAG TCGGAAGACT GGGCCTTTCG TTTTATCTGT
2581 TGTTTGTGCG TGAACGCTCT CCTGAGTAGG ACAAATCCGC CGGAGCGGA TTTGAACGTT
2641 GTGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCCCG CATAACTGC CAGGCATCAA
2701 ACTAAGCAGA AGGCCATC

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FIGURE 11B

**Figure 1A: Cloning Sites of the Entry Vector pENTR3C (reading frame C)**

Int	attL1		DraI		XmnI		SalI		BamHI								
TTG	TAC	AAA	AAA	GCA	GGC	TCT	TTA	AAG	GAA	CCA	ATT	CAG	TCG	ACT	GGA	TCC	GGT
AAC	ATG	TTT	TTT	CGT	CCG	AGA	AAT	TTC	CTT	GGT	TAA	GTC	AGC	TGA	CCT	AGG	CCA
Leu	Tyr	Lys	Lys	Ala	Gly	Ser	Leu	Lys	Glu	Pro	Ile	Gln	Ser	Thr	Gly	Ser	Gly

KpnI	EcoRI		PvuI		EcoRI		NotI		XhoI		EcoRV	XbaI				
AAC	GAA	TTC	GAT	GCG	--	ccdB	--G	AAT	TCG	CGG	CCG	CAC	TCG	AGA	TAT	CTA
TGG	CTT	AAG	CTA	GCG			C	TTA	AGC	GCC	GGC	GTG	AGC	TCT	ATA	GAT
Thr	Glu	Phe					Asn	Ser	Arg	Pro	His	Ser	Arg	Tyr	Leu	

attL2	Int						
GAC	CCA	GCT	TTC	TTG	TAC	AAA	G
CTG	GGT	CGA	AAG	AAC	ATG	TTT	C
Asp	Pro	Ala	Phe	Leu	Tyr	Lys	

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## pENTR3C 2723 bp

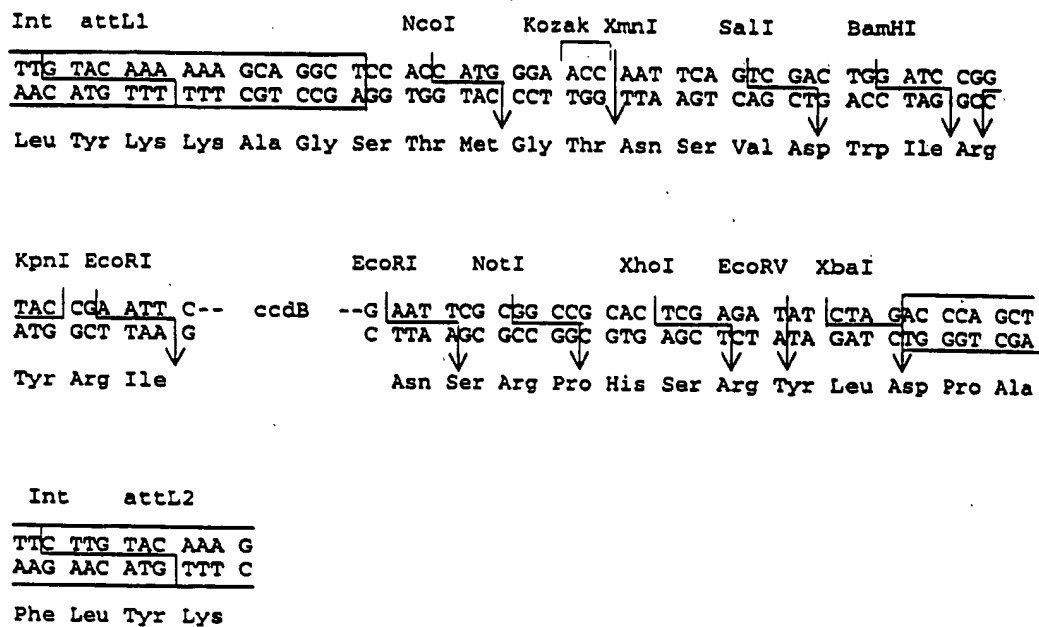
<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
67..166	attL1
327..632	ccdB
661..760	attL2
883..1692	KmR
1797..2370	ori

```

1 CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGA CTGATGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTCTTT AAAGGAACCA
181 ATTCAGTCGA CTGGATCCGG TACCGAATTC GATCGCTTAC TAAAAGCCAG ATAACAGTAT
241 GCGTATTTGC GCGCTGATTT TTGCGGTATA AGAATATATA CTGATATGTA TACCCGAAGT
301 ATGTCAAAAA GAGGTGTGCT TCTAGAATGC AGTTTAAAGT TTACACCTAT AAAAGAGAGA
361 GCCGTTATCG TCTGTTTGTG GATGTACAGA GTGATATTAT TGACACGCCC GGGCGACGGA
421 TGGTGATCCC CCTGGCCAGT GCACGTCTGC TGTCAGATAA AGTCTCCCGT GAACTTTACC
481 CGGTGGTGCA TATCGGGGAT GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC
541 CGGTCTCCGT TATCGGGGAA GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA
601 ACGCCATTAA CCTGATGTTT TGGGGAATAT AGAATTCGCG GCCGCACTCG AGATATCTAG
661 ACCCAGCTTT CTTGTACAAA GTTGGCATT TAAGAAAGCA TTGCTTATCA ATTTGTTGCA
721 ACGAACAGGT CACTATCAGT CAAAATAAAA TCATTATTTG CCATCCAGCT GCAGCTCTGG
781 CCCGTGTCTC AAAATCTCTG ATGTTACATT GCACAAGATA AAAATATATC ATCATGAACA
841 ATAAACTGT CTGCTTACAT AAACAGTAAT ACAAGGGGTG TTATGAGCCA TATTCAACGG
901 GAAACGTCGA GGCCGCGATT AAATCCAAC ATGGATGCTG ATTTATATGG GTATAAATGG
961 GCTCGCGATA ATGTCGGGCA ATCAGGTGCG ACAATCTATC GCTTGTATGG GAAGCCCGAT
1021 GCGCCAGAGT TGTTTCTGAA ACATGGCAAA GGTAGCGTTG CCAATGATGT TACAGATGAG
1081 ATGGTCAGAC TAAACTGGCT GACGGAATTT ATGCCTCTTC CGACCATCAA GCATTTTATC
1141 CGTACTCCTG ATGATGCATG GTTACTCACC ACTGCGATCC CCGGAAAAAC AGCATTCCAG
1201 GTATTAGAAG AATATCCTGA TTCAGGTGAA AATATTGTTG ATGCGCTGGC AGTGTTCCTG
1261 CGCCGGTTGC ATTCGATTCC TGTTTGTAAT TGTCCTTTTA ACAGCGATCG CGTATTTCTG
1321 CTCGCTCAGG CGCAATCACG AATGAATAAC GGTTTGGTTG ATGCGAGTGA TTTTGATGAC
1381 GAGCGTAATG GCTGGCCTGT TGAACAAGTC TGGAAAGAAA TGCATAAACT TTTGCCATTC
1441 TCACCGGATT CAGTCGTCAC TCATGGTGAT TTCTCACTTG ATAACCTTAT TTTTGACGAG
1501 GGGAAATTAA TAGGTTGTAT TGATGTTGGA CGAGTCGGAA TCGCAGACCG ATACCAGGAT
1561 CTTGCGATCC TATGGAACTG CCTCGGTGAG TTTTCTCCTT CATTACAGAA ACGGCTTTTT
1621 CAAAAATATG GTATTGATAA TCCTGATATG AATAAATTGC AGTTTCATTG GATGCTCGAT
1681 GAGTTTTTCT AATCAGAATT GGTAAATTGG TTGTAACATT ATTCAGATTG GGCCCCGTTT
1741 CACTGAGCGT CAGACCCCGT AGAAAAATC AAAGGATCTT CTTGAGATCC TTTTTTCTG
1801 CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG
1861 GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACCTGGT TCAGCAGAGC GCAGATACCA
1921 AATACTGTTT TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGACCCG
1981 CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG
2041 TGTCTTACCG GGTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA
2101 ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC
2161 CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT
2221 CCGGTAAGCG GCAGGGTCCG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC
2281 TGGTATCTTT ATAGTCTCTG CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGA
2341 TGCTCGTCAG GGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGCCTT TTTACGGTTC
2401 CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTCTCTG CGTTATCCCC TGATTCTGTG
2461 GATAACCGTA TTACCGCTAG CATGGATCTC GGGGACGTCT AACTACTAAG CGAGAGTAGG
2521 GAACTGCCAG GCATCAAATA AAACGAAAGG CTCAGTCGGA AGACTGGGCC TTTGTTTTTA
2581 TCTGTTGTTT GTCGGTGAAC GCTCTCTCTG GTAGGACAAA TCCGCCGGGA GCGGATTTGA
2641 ACGTTGTGAA GCAACGGCCC GGAGGGTGGC GGGCAGGACG CCCGCCATAA ACTGCCAGGC
2701 ATCAAATAA GCAGAAGGCC ATC

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FIGURE 12B

**Figure 13A: Cloning Sites of the Entry Vector pENTR4 :**

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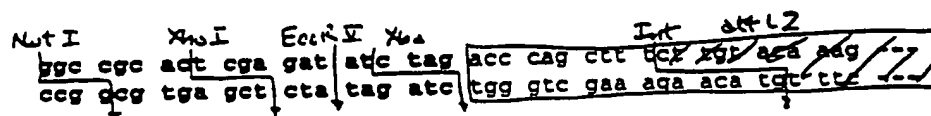
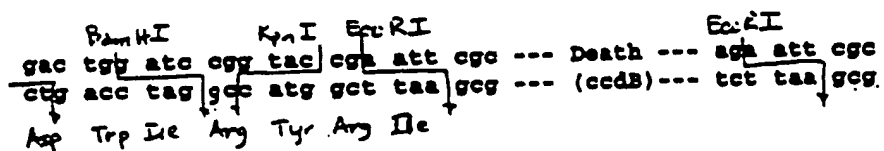
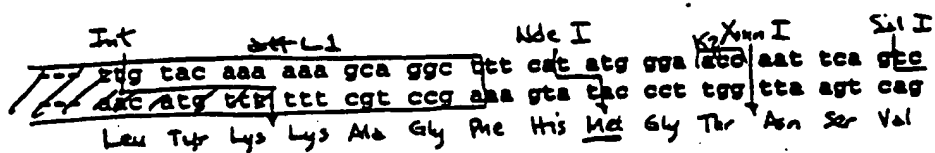
## pENTR4 2720 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>	
67..166		attL1	
324..629		ccdB	
658..757		attL2	
880..1689		KmR	
1794..2367		ori	
1	CTGACGGATG GCCTTTTTGC GTTCTACAA	ACTCTTCCTG	TTAGTTAGTT ACTTAAGCTC
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG	ATAGTGACCT	GTTCGTTGCA ACAAATTGAT
121	AAGCAATGCT TTTTATAAT GCCAACTTTG	TACAAAAAAG	CAGGCTCCAC CATGGGAACC
181	AATTCAGTCG ACTGGATCCG GTACCGAATT	CGCTTACTAA	AAGCCAGATA ACAGTATGCG
241	TATTTGCGCG CTGATTTTTG CGGTATAAGA	ATATATACTG	ATATGTATAC CCGAAGTATG
301	TCAAAAAGAG GTGTGCTTCT AGAATGCAGT	TTAAGGTTTA	CACCTATAAA AGAGAGAGCC
361	GTTATCGTCT GTTTGTGGAT GTACAGAGTG	ATATTATTGA	CACGCCCGGG CGACGGATGG
421	TGATCCCCCT GGCCAGTGCA CGTCTGCTGT	CAGATAAAGT	CTCCCGTGAA CTTTACCCGG
481	TGGTGCATAT CGGGGATGAA AGCTGGCGCA	TGATGACCAC	CGATATGGCC AGTGTGCCGG
541	TCTCCGTTAT CGGGGAAGAA GTGGCTGATC	TCAGCCACCG	CGAAAATGAC ATCAAAAACG
601	CCATTAACCT GATGTTCTGG GGAATATAGA	ATTCGCGGCC	GCACTCGAGA TATCTAGACC
661	CAGCTTTCTT GTACAAAGTT GGCATTATAA	GAAAGCATTG	CTTATCAATT TGTGTCAACG
721	AACAGGTCAC TATCAGTCAA AATAAAATCA	TTATTGCGCA	TCCAGCTGCA GCTCTGGCCC
781	GTGTCTCAAA ATCTCTGATG TTACATTGCA	CAAGATAAAA	ATATATCATC ATGAACAATA
841	AAACTGTCTG CTTACATAAA CAGTAATACA	AGGGGTGTTA	TGAGCCATAT TCAACGGGAA
901	ACGTCGAGGC CGCGATTAAA TTCCAACATG	GATGCTGATT	TATATGGGTA TAAATGGGCT
961	CGCGATAATG TCGGGCAATC AGGTGCGACA	ATCTATCGCT	TGTATGGGAA GCCCAGTGCG
1021	CCAGAGTTGT TTCTGAAACA TGGCAAAGGT	AGCGTTGCCA	ATGATGTTAC AGATGAGATG
1081	GTCAAGCTAA ACTGGCTGAC GGAATTTATG	CCTCTTCCGA	CCATCAAGCA TTTTATCCGT
1141	ACTCCTGGTG ATGCATGGTT ACTCACCCT	GCGATCCCCG	GAAAAACAGC ATTCCAGGTA
1201	TTAGAAGAAT ATCCTGATTC AGGTGAAAAT	ATTGTTGATG	CGCTGGCAGT GTTCTGCGC
1261	CGGTTGCATT CGATTCTGTG TTGTAATTGT	CCTTTTAACA	GCGATCGCGT ATTTCTGCTC
1321	GCTCAGGCGC AATCACGAAT GAATAACGGT	TTGGTTGATG	CGAGTGATTT TGATGACCAG
1381	CGTAATGGCT GGCCTGTTGA ACAAGTCTGG	AAAGAAATGC	ATAAACTTTT GCCATTCTCA
1441	CCGGATTTCAG TCGTCACTCA TGGTGATTTC	TCACTTGATA	ACCTTATTTT TGACGAGGGG
1501	AAATTAATAG GTTGTATTGA TGTGGACGA	GTCGGAATCG	CAGACCGATA CCAGGATCTT
1561	GCCATCCTAT GGAAGTGCCT CGGTGAGTTT	TCTCCTTCAT	TACAGAAACG GCTTTTTCAA
1621	AAATATGGTA TTGATAATCC TGATATGAAT	AAATTGCAGT	TTCATTTGAT GCTCGATGAG
1681	TTTTTCTAAT CAGAATTGGT TAATTGGTTG	TAACATTATT	CAGATTGGGC CCCGTTCCAC
1741	TGAGCGTCAG ACCCGGTAGA AAAGATCAAA	GGATCTTCTT	GAGATCCTTT TTTTCTGCGC
1801	GTAATCTGCT GCTTGCAAAC AAAAAAACCA	CCGCTACCAG	CGGTGGTTTG TTTGCCGGAT
1861	CAAGAGCTAC CAACTCTTTT TCCGAAGGTA	ACTGGCTTCA	GCAGAGCGCA GATACCAAT
1921	ACTGTTCTTC TAGTGTAGCC GTAGTTAGGC	CACCACTTCA	AGAACTCTGT AGCACC GCCT
1981	ACATACCTCG CTCTGCTAAT CCTGTTACCA	GTGGCTGCTG	CCAGTGGCGA TAAGTCGTGT
2041	CTTACCGGGT TGGACTCAAG ACGATAGTTA	CCGGATAAGG	CGCAGCGGTC GGGCTGAACG
2101	GGGGGTTTCG GCACACAGCC CAGCTTGGAG	CGAACGACCT	ACACCGAACT GAGATACCTA
2161	CAGCGTGAGC TATGAGAAAG CGCCACGCTT	CCCGAAGGGA	GAAAGGCGGA CAGGTATCCG
2221	GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC	ACGAGGGAGC	TTCCAGGGGG AAACGCCTGG
2281	TATCTTTATA GTCCTGTCGG GTTTCGCCAC	CTCTGACTTG	AGCGTCGATT TTTGTGATGC
2341	TCGTCAGGGG GCGGAGCCT ATGGAAAAAC	GCCAGCAACG	CGGCCTTTTT ACGGTTCTCTG
2401	GCCTTTTGCT GGCCTTTTGC TCACATGTTT	TTTCTGCGT	TATCCCCTGA TTCTGTGGAT
2461	AACCGTATTA CCGCTAGCAT GGATCTCGGG	GACGTCTAAC	TACTAAGCGA GAGTAGGGAA
2521	CTGCCAGGCA TCAAATAAAA CGAAAGGCTC	AGTCGGAAGA	CTGGGCCTTT CGTTTTATCT
2581	GTTGTTTGTC GGTGAACGCT CTCCTGAGTA	GGACAAATCC	GCCGGGAGCG GATTGTAACG
2641	TTGTGAAGCA ACGGCCCGGA GGGTGGCGGG	CAGGACGCCC	GCCATAAACT GCCAGGCATC
2701	AAACTAAGCA GAAGGCCATC		

FIGURE 13B

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Figure 14A: Cloning sites of the Entry Vector pENTR5



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## pENTR5 2720 bp

Location (Base Nos.)	Gene Encoded
67..166	attL1
324..629	ccdB
658..757	attL2
880..1689	KmR
1794..2367	ori

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1 CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTTTCA TATGGGAACC
181 AATTCAGTCG ACTGGATCCG GTACCGAATT CGCTTACTAA AAGCCAGATA ACAGTATGCG
241 TATTTGCGCG CTGATTTTTG CGGTATAAGA ATATATACTG ATATGTATAC CCGAAGTATG
301 TCAAAAAGAG GTGTGCTTCT AGAATGCAGT TTAAGGTTTA CACCTATAAA AGAGAGAGCC
361 GTTATCGTCT GTTTGTGGAT GTACAGAGTG ATATTATTGA CACGCCCGGG CGACGGATGG
421 TGATCCCCCT GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA CTTTACCCGG
481 TGGTGCAATAT CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC AGTGTGCCGG
541 TCTCCGTTAT CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC ATCAAAAACG
601 CCATTAACTT GATGTTCTGG GGAATATAGA ATTCCGCGCC GCACTCGAGA TATCTAGACC
661 CAGCTTTCTT GTACAAAGTT GGCATTATAA GAAAGCATTG CTTATCAATT TGTGCAACG
721 AACAGGTCAC TATCAGTCAA AATAAAATCA TTATTTGCCA TCCAGCTGCA GCTCTGGCCC
781 GTGTCTCAAA ATCTCTGATG TTACATTGCA CAAGATAAAA ATATATCATC ATGAACAATA
841 AAAGTGTCTG CTTACATAAA CAGTAATACA AGGGGTGTTA TGAGCCATAT TCAACGGGAA
901 ACGTCGAGGC CGCGATTAAA TTCCAACATG GATGCTGATT TATATGGGTA TAAATGGGCT
961 CGCGATAATG TCGGGCAATC AGGTGCGACA ATCTATCGCT TGTATGGGAA GCCCGATGCG
1021 CCAGAGTTGT TTCTGAAACA TGGCAAAGGT AGCGTTGCCA ATGATGTTAC AGATGAGATG
1081 GTCAGACTAA ACTGGCTGAC GGAATTTATG CCTCTTCCGA CCATCAAGCA TTTTATCCGT
1141 ACTCCTGATG ATGCATGGTT ACTCACCCT GCGATCCCCG GAAAAACAGC ATTCCAGSTA
1201 TTAGAAGAAT ATCCTGATTC AGGTGAAAAA ATTGTTGATG CGCTGGCAGT GTTCTGCGC
1261 CGGTTGCATT CGATTCTGTG TTGTAATTGT CCTTTTAAAC GCGATCGCGT ATTTCTGTCT
1321 GCTCAGCGCG AATCACGAAT GAATAACGGT TTGGTTGATG CGAGTGATTT TGATGACGAG
1381 CGTAATGGCT GGCCTGTTGA ACAAGTCTGG AAAGAAATGC ATAAACTTTT GCCATTCTCA
1441 CCGGATTGAG TCCTCACTCA TGGTGATTTT TCACTTGATA ACCTTATTTT TGACGAGGGG
1501 AAATTAATAG GTTGATTTGA TGTGGAACGA GTCGGAATCG CAGACCGATA CCAGGATCTT
1561 GCCATCCCTAT GGAAGTGCCT CGGTGAGTTT TCTCCTTCAT TACAGAAACG GCTTTTTCAA
1621 AAATATGGTA TTGATAATCC TGATATGAAT AAATTGCACT TTCATTGATG GCTCGATGAG
1681 TTTTCTAAT CAGAATTGGT TAATTGGTTG TAACATTATT CAGATTGGGC CCCGTTCCAC
1741 TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC
1801 GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CCGTGCTTTG TTGCGCGGAT
1861 CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT
1921 ACTGTTCTTC TAGTGATGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT
1981 ACATACCTCG CTCTGCTAAT CCGTTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT
2041 CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG
2101 GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA
2161 CAGCGTGAGC TATGAGAAAG CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG
2221 GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGGAG TTCCAGGGGG AAACGCCTGG
2281 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC
2341 TCGTCAGGGG GCGCGAGCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTT ACGGTTCTCT
2401 GCCTTTTGCT GGCCTTTTGC TCACATGTTT TTTCTGCGT TATCCCTGTA TTCTGTGGAT
2461 AACCCTATTA CCGCTAGCAT GGATCTCGGG GACGTCTAAC TACTAAGCGA GAGTAGGGAA
2521 CTGCCAGGCA TCGAATAAAA CGAAAGGCTC AGTCGGAAGA CTGGGCCTTT CGTTTTATCT
2581 GTTGTGTTGC GGTGAACGCT CTCCTGAGTA GGACAAATCC GCCGGGAGCG GATTTGAACG
2641 TTGTGAAGCA ACGGCCCCGA GGGTGGCGGG CAGGACGCCC GCCATAAACT GCCAGGCATC
2701 AAATAAGCA GAAGGCCATC

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FIGURE 14B

Figure 15A: Cloning sites of the Entry Vector pENTR6

Int                      attL1                      Sph I                      Kpn I                      Xba I                      Sph I  
~~--- tgg tac aaa aaa gca ggc tgc atg cga acc aat tca gcc~~  
~~--- aac atg ttt ttt cgt ccg atg taa ggt tgg tta agt cag~~  
 Leu Tyr Lys Lys Ala Gly Cys Met Arg Thr Asn Ser Val

BamH I                      Kpn I                      EpeRI                      EpeRI  
 gac tgg atc cgg tac cga att cgc --- Death --- aga att cgc  
 cgg acc tag gct atg gct taa ggc --- (codB) --- tct taa ggc  
 Asp Trp Ile Arg Tyr Arg Ile

Not                      Xba I                      EpeRI                      Xba I                      Int                      attL2  
 ggc cgc act cga gat atc tag acc cag att tgc tgc aca aag ---  
 ccg ggc tga gct cta tag atc tgg gtc gaa aga aca tgc tcc ---



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## pENTR6 2717 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
321..626		ccdB
655..754		attL2
877..1686		KmR
1791..2364		ori

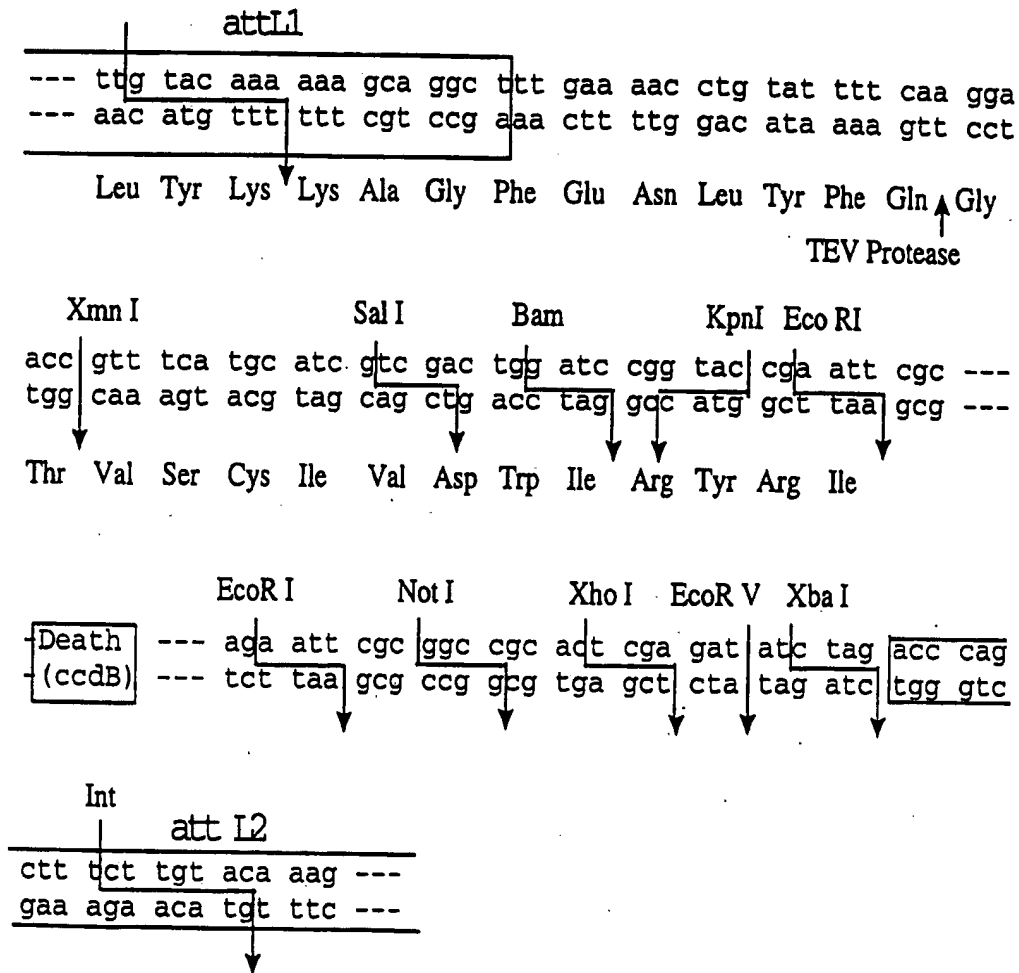
  

1	CTGACGGATG	GCCTTTTTCG	GTTTCTACAA	ACTCTTCCTG	TTAGTTAGTT	ACTTAAGCTC
61	GGGCCCCAAA	TAATGATTTT	ATTTTGACTG	ATAGTGACCT	GTTGCGTTGCA	ACAAATTGAT
121	AAGCAATGCT	TTTTTATAAT	GCCAACTTTG	TACAAAAAAG	CAGGCTGCGT	GCGAACCAAT
181	TCAGTCGACT	GGATCCGGTA	CCGAATTCGC	TTACTAAAAG	CCAGATAACA	GTATGCGTAT
241	TTGCGCGCTG	ATTTTTCGGG	TATAAGAATA	TATACTGATA	TGTATACCCG	AAGTATGTCA
301	AAAAGAGGTG	TGCTTCTAGA	ATGCAGTTTA	AGGTTTACAC	CTATAAAAGA	GAGAGCCGTT
361	ATCGTCTGTT	TGTGGATGTA	CAGAGTGATA	TTATTGACAC	GCCCCGGCGA	CGGATGGTGA
421	TCCCCCTGGC	CAGTGCACGT	CTGCTGTCAG	ATAAAGTCTC	CCGTGAACCT	TACCCGGTGG
481	TGCATATCGG	GGATGAAAGC	TGGCGCATGA	TGACCACCGA	TATGGCCAGT	GTGCCGCTCT
541	CCGTTATCGG	GGAAGAAGTG	GCTGATCTCA	GCCACCGCGA	AAATGACATC	AAAAACGCCA
601	TTAACCTGAT	GTTCTGGGGA	ATATAGAATT	CGCGGCCGCA	CTCGAGATAT	CTAGACCCAG
661	CTTCTTGTA	CAAAGTTGGC	ATTATAAGAA	AGCATTGCTT	ATCAATTTGT	TGCAACGAAC
721	AGGTCACTAT	CAGTCAAAAT	AAAATCATT	TTTGCCATCC	AGCTGCAGCT	CTGGCCCGTG
781	TCTCAAAATC	TCTGATGTTA	CATTGCACAA	GATAAAAATA	TATCATCATG	ACCAATAAAA
841	CTGTCTGCTT	ACATAAACAG	TAATACAAGG	GGTGTATGA	GCCATATTCA	ACGGGAAACG
901	TCGAGGCCGC	GATTAAATTC	CAACATGGAT	GCTGATTAT	ATGGGTATAA	ATGGGCTCGC
961	GATAATGTCG	GGCAATCAGG	TGCGACAATC	TATCGCTTGT	ATGGGAAGCC	CGATGCGCCA
1021	GAGTTGTTTC	TGAAACATGG	CAAAGGTAGC	GTTGCCAATG	ATGTTACAGA	TGAGATGGTC
1081	AGACTAAACT	GGCTGACGGA	ATTTATGCCT	CTTCCGACCA	TCAAGCATT	TATCCGTACT
1141	CCTGATGATG	CATGGTTACT	CACCACTGCG	ATCCCCGGAA	AAACAGCATT	CCAGGTATTA
1201	GAAGAATATC	CTGATTCAGG	TGAAAATATT	GTTGATGCGC	TGGCAGTGT	CCTGCGCCGG
1261	TTGCATTGCA	TTCCTGTTTG	TAATTGTCTT	TTTAACAGCG	ATCGCGTATT	TCGTCTCGCT
1321	CAGGCGCAAT	CACGAATGAA	TAACGGTTTG	GTTGATGCGA	GTGATTTTGA	TGACGAGCGT
1381	AATGGCTGGC	CTGTTGAACA	AGTCTGGAAA	GAAATGCATA	AACTTTTGCC	ATTCTCACCG
1441	GATTCAGTCG	TCACTCATGG	TGATTTCTCA	CTTGATAACC	TTATTTTGA	CGAGGGGAAA
1501	TTAATAGGTT	GTATTGATGT	TGGACGAGTC	GGAATCGCAG	ACCGATACCA	GGATCTTGCC
1561	ATCCTATGGA	ACTGCCTCGG	TGAGTTTCTT	CCTTCATTAC	AGAAACGGCT	TTTTCAAAAA
1621	TATGGTATTG	ATAATCTGTA	TATGAATAAA	TTGCAGTTTC	ATTTGATGCT	CGATGAGTTT
1681	TTCTAATCAG	AATTGGTTAA	TTGGTTGTAA	CATTATTCAG	ATTGGGCCCC	GTTCCACTGA
1741	GCGTCAGACC	CCGTAGAAAA	GATCAAAGGA	TCTTCTTGAG	ATCCTTTTTT	TCTGCGCGTA
1801	ATCTGCTGCT	TGCAAAACAA	AAAACCACCG	CTACCAGCGG	TGGTTTGT	GCCGGATCAA
1861	GAGCTACCAA	CTCTTTTTC	GAAGGTAAC	GGCTTCAGCA	GAGCGCAGAT	ACCAAATACT
1921	GTTCTTCTAG	TGTAGCCGTA	GTTAGGCCAC	CACCTCAAGA	ACTCTGTAGC	ACCGCCTACA
1981	TACCTCGCTC	TGCTAATCCT	GTTACCACTG	GCTGCTGCCA	GTGGCGATAA	GTCGTGTCTT
2041	ACCGGGTTGG	ACTCAAGACG	ATAGTTACCG	GATAAGGCGC	AGCGGTCGGG	CTGAACGGGG
2101	GGTTCGTGCA	CACAGCCCAG	CTTGGAGCGA	ACGACCTACA	CCGAACCTGAG	ATACCTACAG
2161	CGTGAGCTAT	GAGAAAGCGC	CACGCTTCCC	GAAGGGAGAA	AGGCGGACAG	GTATCCGGTA
2221	AGCGGCAGGG	TCGGAACAGG	AGAGCGCACG	AGGGAGCTTC	CAGGGGGAAA	CGCCTGCTAT
2281	CTTTATAGTC	CTGTCGGGTT	TCGCCACCTC	TGACTTGAGC	GTGATTTTTT	GTGATGCTCG
2341	TCAGGGGGGG	GGAGCCTATG	GAAAAACGCC	AGCAACGCGG	CCTTTTACG	GTTCTTGCGC
2401	TTTTGCTGGC	CTTTTGCTCA	CATGTTCTTT	CCTGCGTTAT	CCCCTGATT	TGTGGATAAC
2461	CGTATTACCG	CTAGCATGGA	TCTCGGGGAC	GTCTAACTAC	TAAGCGAGAG	TAGGGAACCTG
2521	CCAGGCATCA	AATAAACGGA	AAGGCTCAGT	CGGAAGACTG	GGCCTTTCGT	TTTATCTGTT
2581	GTTTGTGCGT	GAACGCTCTC	CTGAGTAGGA	CAAATCCGCC	GGGAGCGGAT	TTGAACGTTG
2641	TGAAGCAACG	GCCCCGAGGG	TGGCGGGCAG	GACGCCCCGC	ATAAACTGCC	AGGCATCAAA
2701	CTAAGCAGAA	GGCCATC				

Figure 15B

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Figure 16A: Cloning sites of the Entry Vector pENTR7



## pENTR7 2738 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
342..647		ccdB
676..775		attL2
898..1707		KmR
1812..2385		ori
1	CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCCAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT	
181	TTTCAAGGAA CCGTTTCATG CATCGTCGAC TGGATCCGGT ACCGAATTCG CTTACTAAAA	
241	GCCAGATAAC AGTATGCGTA TTTGCGCGCT GATTTTTCG GTATAAGAAT ATATACTGAT	
301	ATGTATACCC GAAGTATGTC AAAAAGAGGT GTGCTTCTAG AATGCAGTTT AAGGTTTACA	
361	CCTATAAAAG AGAGAGCCGT TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA	
421	CGCCCGGGCG ACGGATAGTG ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT	
481	CCCGTGAAC TACCCCGTG GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG	
541	ATATGGCCAG TGTGCCGGTC TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG	
601	AAAATGACAT CAAAAACGCC ATTAACCTGA TGTTCTGGGG AATATAGAAT TCGCGGCCGC	
661	ACTCGAGATA TCTAGACCCA GCTTCTTGT ACAAAGTTGG CATTATAAGA AAGCATTGCT	
721	TATCAATTG TTGCAACGAA CAGGTCATA TCAGTCAAAA TAAATCATT ATTTGCCATC	
781	CAGCTGCAGC TCTGGCCCGT GTCTCAAAAT CTCTGATGTT ACATGACACA AGATAAAAAT	
841	ATATCATCAT GAACAATAAA ACTGTCTGCT TACATAAACA GTAATACAAG GGGTGTATG	
901	AGCCATATTC AACGGGAAAC GTCGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA	
961	TATGGGTATA AATGGGCTCG CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGCTTG	
1021	TATGGGAAGC CCGATGCGCC AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT	
1081	GATGTTACAG ATGAGATGGT CAGACTAAAC TGGCTGACGG AATTTATGCC TCTTCCGACC	
1141	ATCAAGCATT TTATCCGTAC TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCCGGA	
1201	AAAACAGCAT TCCAGGTATT AGAAGAATAT CCTGATTGAG GTGAAAATAT TGTTGATGCG	
1261	CTGGCAGTGT TCCTGCGCCG GTTGCAATCG ATTCTGTTT GTAATTGTCC TTTTAACAGC	
1321	GATCGCGTAT TTCGTCTCGC TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTGATGCG	
1381	AGTGATTTTG ATGACGAGCG TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT	
1441	AAACTTTTGC CATTCTCACC GGATTGAGTC GTCACCTATG GTGATTTCTC ACTTGATAAC	
1501	CTTATTTTTC ACGAGGGGAA ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA	
1561	GACCGATACC AGGATCTTGC CATCCTATGG AACTGCCCTCG GTGAGTTTTC TCCTTCATTA	
1621	CAGAAACGGC TTTTCAAAA ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT	
1681	CATTTGATGC TCGATGAGTT TTTCTAATCA GAATTGGTTA ATTGGTTGTA ACATTATTCA	
1741	GATTGGGCCC CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA	
1801	GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAACAAA AAAAACCACC GCTACCAGCG	
1861	GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC	
1921	AGAGCGCAGA TACCAAATAC TGTTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG	
1981	AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC	
2041	AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG	
2101	CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC	
2161	ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA	
2221	AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT	
2281	CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCCGGT TTCGCCACCT CTGACTTGAG	
2341	CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG	
2401	GCCTTTTAC GGTTCCTGGC CTTTGTCTGG CCTTTTGTCT ACATGTTCTT TCTGCGTTA	
2461	TCCCCTGATT CTGTGGATAA CCGTATTACC GCTAGCATGG ATCTCGGGGA CGTCTAATA	
2521	CTAAGCGAGA GTAGGGAAC TCCAGGCATC AAATAAAACG AAAGGCTCAG TCGGAAGACT	
2581	GGGCCTTTCG TTTTATCTGT TGTTGTCTCG TGAACGCTCT CCTGAGTAGG ACAAATCCGC	
2641	CGGGAGCGGA TTTGAACGTT GTGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCCCG	
2701	CATAAACTGC CAGGCATCAA ACTAAGCAGA AGGCCATC	

Figure 16B

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Figure 17A: Cloning Sites of the ENTRY Vector: pENTRY8

Int attC1

```

// 5'---ttg tac aaa aaa gca ggc ttt gaa aac ctg tat ttt caa gga
// 3'---aac atg aaa aaa cgt gcc aaa ctt ttg gag ata aaa gtt cct

```

Leu Tyr Lys Lys Ala Gly Phe Glu Asn Leu Tyr Phe Gln Gly

TEV Protease

NotI AatII SalI BamHI KpnI EcoRI

```

acc atg gac cta gcc gac tgg atc cgg tac cga att cgc ---
tgg tac ctg gat cag cgg acc tag gcc atg gct taa gcg ---

```

Thr Met Asp Leu Val Asp Trp Ile Arg Tyr Arg Ile

EcoRI NotI XhoI EcoRV XbaI attC

```

Death --- aga att cgc ggc cgc act cga gat atc tag acc cag
--- tct taa gcg cgc gcc tga gct cta tag atc tgg gtc

```

Int

```

ctt tct tct aga tag ---
gaa aga aca tgc ttc ---

```

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## pENTR8 2735 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
339..644		ccdB
673..772		attL2
895..1704		KmR
1809..2382		ori

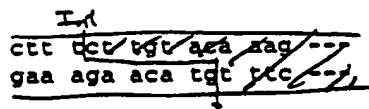
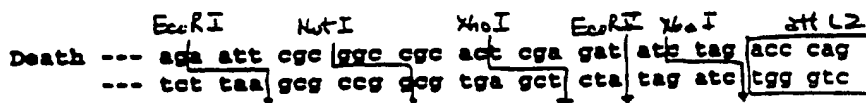
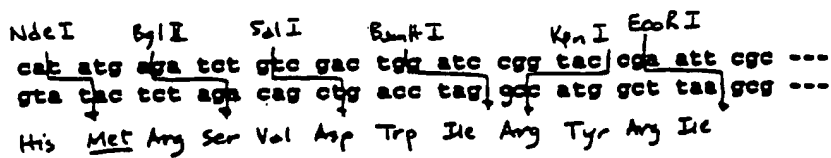
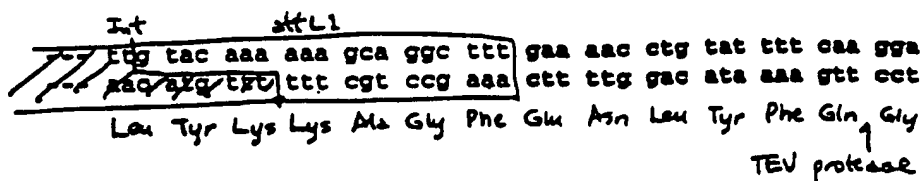
  

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61	GGGCCCCAAA	TAATGATTTT	ATTTTGACTG	ATAGTGACCT	GTTCGTTGCA	ACAAATTGAT
121	AAGCAATGCT	TTTTTATAAT	GCCAACTTTG	TACAAAAAAG	CAGGCTTTGA	AAACCTGTAT
181	TTTCAAGGAA	CCATGGACCT	AGTCGACTGG	ATCCGGTACC	GAATTCGCTT	ACTAAAAGCC
241	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT	TTTGCGGGTA	TAAGAATATA	TACTGATATG
301	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG	CTTCTAGAAT	GCAGTTTAAAG	GTTTACACCT
361	ATAAAAGAGA	GAGCCGTTAT	CGTCTGTTTG	TGGATGTACA	GAGTGATATT	ATTGACACGC
421	CCGGGCGACG	GATAGTGATC	CCCCTGGCCA	GTGCACGTCT	GCTGTCAGAT	AAAGTCTCCC
481	GTGAACTTTA	CCCGGTGGTG	CATATCGGGG	ATGAAAGCTG	GCGCATGATG	ACCACCGATA
541	TGGCCAGTGT	GCCGGTCTCC	GTTATCGGGG	AAGAAAGTGGC	TGATCTCAGC	CACCCGCAAA
601	ATGACATCAA	AAACGCCATT	AACCTGATGT	TCTGGGGAAT	ATAGAATTCTG	CGGCCGCACT
661	CGAGATATCT	AGACCCAGCT	TTCTTGTACA	AAGTTGGCAT	TATAAGAAAG	CATTGCTTAT
721	CAATTTGTTG	CAACGAACAG	GTCACATCA	GTCAAAATAA	AATCATTATT	TGCCATCCAG
781	CTGCAGCTCT	GGCCCGTGTC	TCAAAATCTC	TGATGTTACA	TGCAACAAGA	TAAAAATATA
841	TCATCATGAA	CAATAAAACT	GTCTGCTTAC	ATAAACAGTA	ATACAAGGGG	TGTTATGAGC
901	CATATTCAAC	GGGAAACGTC	GAGGCCGCGA	TTAAATTCCA	ACATGGATGC	TGATTTATAT
961	GGGTATAAAT	GGGCTCGCGA	TAATGTCGGG	CAATCAGGTG	CGACAATCTA	TCGCTTGTAT
1021	GGGAAGCCCC	ATGCGCCAGA	GTTGTTTCTG	AAACATGGCA	AAGGTAGCGT	TGCCAATGAT
1081	GTTACAGATG	AGATGGTCAG	ACTAACTGG	CTGACGGAAT	TTATGCCTCT	TCCGACCATC
1141	AAGCATTTTA	TCCGTACTCC	TGATGATGCA	TGGTTACTCA	CCACTGCGAT	CCCCGGAAAA
1201	ACAGCATTCC	AGGTATTAGA	AGAATATCCT	GATTCAGGTG	AAAATATTGT	TGATGCGCTG
1261	GCAGTGTCCC	TGCGCCGGTT	GCATTCGATT	CCTGTTTGTA	ATTGTCCTTT	TAACAGCGAT
1321	CGCGTATTTT	GTCTCGCTCA	GGCGCAATCA	CGAATGAATA	ACGGTTTGGT	TGATGCGAGT
1381	GATTTTGATG	ACGAGCGTAA	TGGCTGGCCT	GTTGAACAAG	TCTGGAAAGA	AATGCATAAA
1441	CTTTTGCCAT	TCTCACCGBA	TTCAGTCGTC	ACTCATGGTG	ATTCTCACT	TGATAACCTT
1501	ATTTTGTACG	AGGGGAAATT	AATAGGTTGT	ATTGATGTTG	GACGAGTCGG	AATCGCAGAC
1561	CGATACCAGG	ATCTTGCCAT	CCTATGGAAC	TGCCTCGGTG	AGTTTTCTCT	TTCAATTACAG
1621	AAACGGCTTT	TTCAAAAATA	TGGTATTGAT	AATCCTGATA	TGAATAAATT	GCAGTTTCAT
1681	TTGATGCTCG	ATGAGTTTTT	CTAATCAGAA	TTGGTTAATT	GGTTGTAACA	TTATTTCAGAT
1741	TGGGCCCCGT	TCCACTGAGC	GTCAGACCCC	GTAAGAAAAG	TCAAAGGATC	TTCTTGAGAT
1801	CCTTTTTCCT	TGCGCGTAAT	CTGCTGCTTG	CAAACAAAAA	AACCACCGCT	ACCAGCGGTG
1861	GTTTGTTCCT	CGGATCAAGA	GCTACCAACT	CTTTTTCCTG	AGGTAACCTG	CTTCAGCAGA
1921	GCGCAGATAC	CAAATACTGT	TCTTCTAGTG	TAGCCGTAGT	TAGGCCACCA	CTTCAAGAAC
1981	TCTGTAGCAC	CGCCTACATA	CCTCGCTCTG	CTAATCCTGT	TACCAGTGGC	TGCTGCCAGT
2041	GGCGATAAGT	CGTGTCTTAC	CGGGTTGGAC	TCAAGACGAT	AGTTACCGGA	TAAGGCGCAG
2101	CGGTGCGGCT	GAACGGGGGG	TTCGTGCACA	CAGCCCAGCT	TGGAGCGAAC	GACCTACACC
2161	GAACTGAGAT	ACCTACAGCG	TGAGCTATGA	GAAAGCGCCA	CGCTTCCCGA	AGGGAGAAAG
2221	GCGGACAGGT	ATCCGGTAAG	CGGCAGGGTC	GGAACAGGAG	AGCGCACGAG	GGAGCTTCCA
2281	GGGGGAAACG	CCTGGTATCT	TTATAGTCCT	GTCGGGTTTC	GCCACCTCTG	ACTTGAGCGT
2341	CGATTTTTGT	GATGCTCGTG	AGGGGGGCGG	AGCCTATGGA	AAAACGCCAG	CAACGCGGCC
2401	TTTTTACGGT	TCCTGGCCTT	TTGTGGCCTT	TTTGCTCACA	TGTTCTTTCC	TGCGTTATCC
2461	CCTGATTCTG	TGGATAACCG	TATTACCGCT	AGCATGGATC	TCGGGGACGT	CTAACTACTA
2521	AGCGAGAGTA	GGGAACGCCC	AGGCATCAAA	TAAAACGAAA	GGCTCAGTCG	GAAGACTGGG
2581	CCTTTCGTTT	TATCTGTTGT	TTGTCGGTGA	ACGCTCTCCT	GAGTAGGACA	AATCCGCCGG
2641	GAGCGGATTT	GAACGTTGTG	AAGCAACGGC	CCGGAGGGTG	GCGGGCAGGA	CGCCCGCCAT
2701	AAACTGCCAG	GCATCAAACT	AAGCAGAAGG	CCATC		

FIGURE 17B

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Figure 18: Cloning sites of the ENTRY Vector pENTRY



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## pENTR9 2735 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
339..644		ccdB
673..772		attL2
895..1704		KmR
1809..2382		ori
1	CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT	
181	TTTCAAGGAC ATATGAGATC TGTCGACTGG ATCCGGTACC GAATTCGCTT ACTAAAAGCC	
241	AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGCGGTA TAAGAATATA TACTGATATG	
301	TATACCCGAA GTATGTCAAA AAGAGGTGTG CTTCTAGAAT GCAGTTTAAG GTTTACACCT	
361	ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA GAGTGATATT ATTGACACGC	
421	CCGGGCGACG GATAGTGATC CCCCTGGCCA GTGCACGCTC GCTGTCAGAT AAAGTCTCCC	
481	GTGAACCTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG GCGCATGATG ACCACCGATA	
541	TGGCCAGTGT GCCGGTCTCC GTTATCGGGG AAGAAAGTGGC TGATCTCAGC CACCGCGAAA	
601	ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT ATAGAATTCG CGGCCGCACT	
661	CGAGATATCT AGACCCAGCT TTCTTGATACA AAGTTGGCAT TATAAGAAAG CATTTGCTTAT	
721	CAATTTGTGT CAACGAACAG GTCACATCA GTCAAAATAA AATCATTATT TGCCATCCAG	
781	CTGCAGCTCT GGCCCGTGTG TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA	
841	TCATCATGAA CAATAAAACT GTCTGCTTAC ATAAACAGTA ATACAAGGGG TGTTATGAGC	
901	CATATTC AAC GGGAAACGTC GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTTATAT	
961	GGGTATAAAT GGGCTCGCGA TAATGTCGGG CAATCAGGTG CGACAATCTA TCGCTTGTAT	
1021	GGGAAGCCCG ATGCGCCAGA GTTGTTTCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT	
1081	GTTACAGATG AGATGGTCAG ACTAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC	
1141	AAGCATTTTA TCCGTACTCC TGATGATGCA TGGTTACTCA CCACTGCGAT CCCCAGAAAA	
1201	ACAGCATTCC AGGTATTAGA AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG	
1261	GCAGTGTCCT TGCGCCGGTT GCATTTCGATT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT	
1321	CGCGTATTTT GTCTCGCTCA GCGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT	
1381	GATTTTGATG ACGAGCGTAA TGGCTGGCCT GTTGAACAAG TCTGGAAGA AATGCATAAA	
1441	CTTTTGCCAT TCTCACC GGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT	
1501	ATTTTGTGACG AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC	
1561	CGATACCAGG ATCTTGCCAT CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG	
1621	AAACGGCTTT TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT	
1681	TTGATGCTCG ATGAGTTTTT CTAATCAGAA TTGGTTAATT GGTGTGAACA TTATTAGAT	
1741	TGGGCCCCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT	
1801	CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG	
1861	GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTCCGA AGGTAACCTG CTTACAGCAG	
1921	GCGCAGATAC CAAATACTGT TCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC	
1981	TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TCGTGCCAGT	
2041	GGCGATAAGT CGTGCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG	
2101	CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC	
2161	GAACGTAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAAG	
2221	GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTTCCA	
2281	GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT	
2341	CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC	
2401	TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC TCGGTTATCC	
2461	CCTGATTCTG TGGATAACCG TATTACCGCT AGCATGGATC TCGGGGACGT CTAACACTA	
2521	AGCGAGAGTA GGGAACTGCC AGGCATCAAA TAAAACGAAA GGCTCAGTCG GAAGACTGGG	
2581	CCTTTCGTTT TATCTGTTGT TTGTCGGTGA ACGTCTCCT GAGTAGGACA AATCCGCCGG	
2641	GAGCGGATTT GAACGTTGTG AAGCAACGGC CCGGAGGGTG GCGGGCAGGA CGCCCGCCAT	
2701	AAACTGCCAG GCATCAAAC AAGCAGAAGG CCATC	

FIGURE 18B

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Figure 19A: Cloning sites of the ENTRY Vector pENTRY10

Int                      attL1                      S.D.                      -12                      Nde

--- tgg tac aaa aaa gca ggc ttc gaa cta agg aaa tac tta cct  
 --- aac atg att ttt cgt ccg aag ctt gat tcc ttt atg aat gta  
 Leu Tyr Lys Lys Ala Gly Phe Glu Leu Arg Lys Tyr Leu His

atg gga acc aat tca gtc gac tgg atc cgg tac cga att cgc ---  
 tac cct tgg tta agt cag cgg acc tag gcp atg gct taa ggc ---  
 Met Gly Thr Asn Ser Val Asp Trp Ile Arg Tyr Arg Ile

EcoRI                      Not                      Xho                      EcoRII                      Xba                      attL2

Death --- aga att cgc ggc cgc act cga gat atc tag acc cag  
 (ccdB) --- tct taa ggc ccg ggc tga gct cta tag atc tgg gtc

Int

ctt tgg agc aca aag  
 gaa aga aca tgc tgc



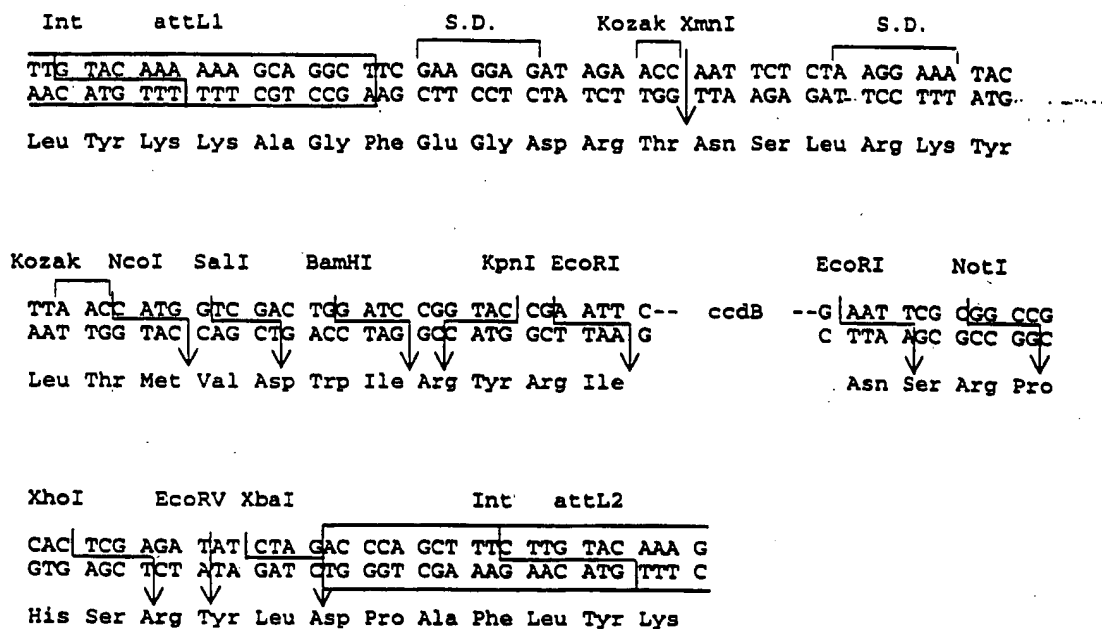
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## pENTR10 2738 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
342..647		ccdB
676..775		attL2
898..1707		KmR
1812..2385		ori
1	CTGACGGATG GCCTTTTTGC GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTTCGA ACTAAGGAAA	
181	TACTTACATA TGGGAACCAA TTCAGTCGAC TGGATCCGGT ACCGAATTCG CTTACTAAAA	
241	GCCAGATAAC AGTATGCGTA TTTGCGCGCT GATTTTTGCG GTATAAGAAT ATATACTGAT	
301	ATGTATACCC GAAGTATGTC AAAAAGAGGT GTGCTTCTAG AATGCAGTTT AAGGTTTACA	
361	CCTATAAAAG AGAGAGCCGT TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA	
421	CGCCCCGGCG ACGGATGGTG ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT	
481	CCCGTGAAC TTTACCCGGTG GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG	
541	ATATGGCCAG TGTGCCGGTC TCCGTTATCG GGGAGAAGT GGCTGATCTC AGCCACCGCG	
601	AAAATGACAT CAAAACGCC ATTAACCTGA TGTCTGGGG AATATAGAAT TCGCGGCCGC	
661	ACTCGAGATA TCTAGACCCA GCTTTCTGT ACAAAGTTGG CATTATAAGA AAGCATTGCT	
721	TATCAATTTG TTGCAACGAA CAGGTCAC TAAGTCAAAA TAAATCATT ATTTGCCATC	
781	CAGCTGCAGC TCTGGCCCGT GTCTCAAAAT CTCTGATGTT ACATTGCACA AGATAAAAAT	
841	ATATCATCAT GAACAATAAA ACTGTCTGCT TACATAAACA GTAATACAAG GGGTGTATG	
901	AGCCATATTC AACGGGAAAC GTCGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA	
961	TATGGGTATA AATGGGCTCG CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGCTTG	
1021	TATGGGAAGC CCGATGCGCC AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT	
1081	GATGTTACAG ATGAGATGGT CAGACTAAAC TGGCTGACGG AATTTATGCC TCTCCGACC	
1141	ATCAAGCATT TTATCCGTAC TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCCGGA	
1201	AAAACAGCAT TCCAGGTATT AGAAGAATAT CCTGATTGAG GTGAAAAATAT TGTGATGCG	
1261	CTGGCAGTGT TCCTGCGCCG GTTGCAATCG ATTCCTGTTT GTAATTGTCC TTTTAACAGC	
1321	GATCGCGTAT TTCGTCTCGC TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTGATGCG	
1381	AGTGATTTTG ATGACGAGCG TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT	
1441	AAACTTTTGC CATTCTCACC GGATTACGTC GTCACTCATG GTGATTTCTC ACTTGATAAC	
1501	CTTATTTTTC ACGAGGGGAA ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA	
1561	GACCGATACC AGGATCTTGC CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA	
1621	CAGAAACGCG TTTTTCAAAA ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT	
1681	CATTTGATGC TCGATGAGTT TTTCTAATCA GAATTGGTTA ATTGGTTGTA ACATTATTCA	
1741	GATTGGGCCC CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA	
1801	GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG	
1861	GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC	
1921	AGAGCGCAGA TACCAAATAC TGTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG	
1981	AACTCTGTAG CACCGCTAC ATACCTCGCT CTGCTAATCC TGTACCAGT GGCTGCTGCC	
2041	AGTGGCGATA AGTCGTGTCT TACCGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG	
2101	CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA GCTTGAGCG AACGACCTAC	
2161	ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA	
2221	AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT	
2281	CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG	
2341	CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG	
2401	GCCTTTTTAC GGTTCCTGGC CTTTTCCTGG CTTTTCCTC ACATGTTCTT TCCTGCGTTA	
2461	TCCCTTGATT CTGTGGATAA CCGTATTACC GCTAGCATGG ATCTCGGGGA CGTCTAACTA	
2521	CTAAGCGAGA GTAGGGAAC GCCAGGCATC GAATAAAACG AAAGGCTCAG TCGGAAGACT	
2581	GGGCCTTTTC TTTTATCTGT TGTGTCGGG TGAACGCTCT CCTGAGTAGG ACAAATCCGC	
2641	CGGGAGCGGA TTTGAACGTT GTGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCCCG	
2701	CATAAACTGC CAGGCATCAA ACTAAGCAGA AGGCCATC	

FIGURE 19B

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**Figure 20A: Cloning Sites of the Entry Vector pENTR11**

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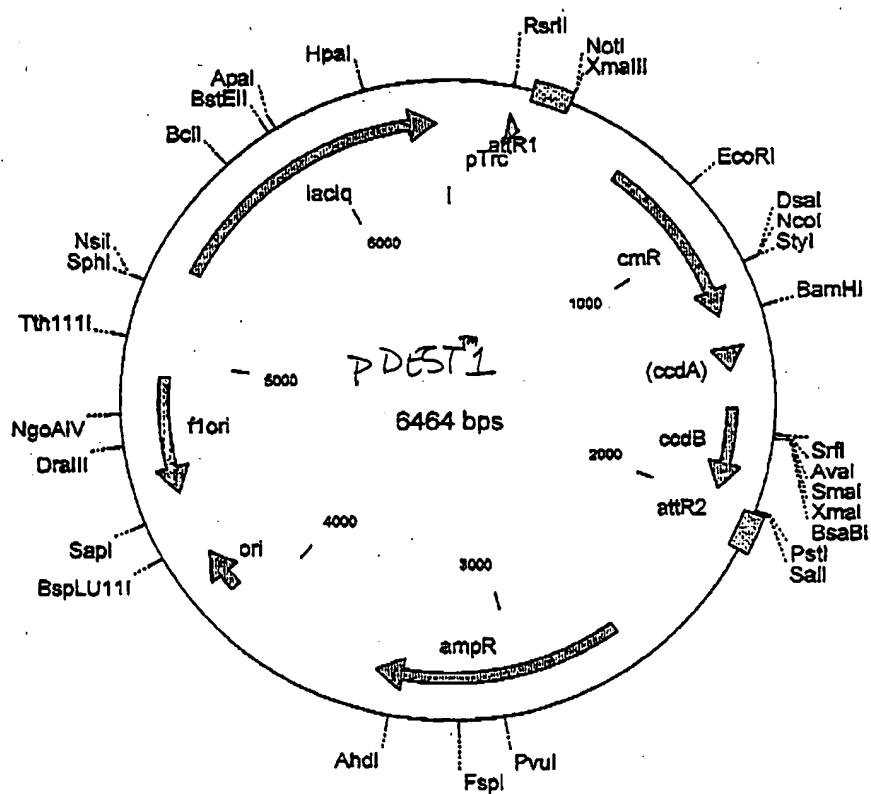
## pENTR11 2744 bp (rotated to position 2578)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>	
67..166		attL1	
348..653		ccdB	
683..781		attL2	
904..1713		KmR	
1818..2391		ori	
1	CTGACGGATG GCCTTTTTGC GTTCTACAA	ACTCTTCCTG TTAGTTAGTT	ACTTAAGCTC
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG	ATAGTGACCT GTTCGTTGCA	ACAAATTGAT
121	AAGCAATGCT TTTTATAAT GCCAACTTTG	TACAAAAAAG CAGGCTTCGA	AGGAGATAGA
181	ACCAATTCTC TAAGGAAATA CTTAACCATG	GTCGACTGGA TCCGGTACCG	AATTCGCTTA
241	CTAAAAGCCA GATAACAGTA TCGGTATTTG	CGCGCTGATT TTTGCGGTAT	AAGAATATAT
301	ACTGATATGT ATACCCGAAG TATGTCAAAA	AGAGGTGTGC TTCTAGAATG	CAGTTTAAAG
361	TTTACACCTA TAAAAGAGAG AGCCGTTATC	GTCTGTTTGT GGATGTACAG	AGTGATATTA
421	TTGACACGCC CGGGCGACGG ATAGTGATCC	CCCTGGCCAG TGCACGCTCG	CTGTCAGATA
481	AAGTCTCCCG TGAACTTTAC CCGGTGGTGC	ATATCGGGGA TGAAAGCTGG	CGCATGATGA
541	CCACCGATAT GGCCAGTGTG CCGTCTCCG	TTATCGGGGA AGAAGTGGCT	GATCTCAGCC
601	ACCGCGAAAA TGACATCAAA AACGCCATTA	ACCTGATGTT CTGGGGAATA	TAGAATTCGC
661	GGCCGCACTC GAGATATCTA GACCCAGCTT	TCTTGTAACA AGTTGGCATT	ATAAGAAAGC
721	ATTGCTTATC AATTTGTGTC AACGAACAGG	TCACTATCAG TCAAAATAAA	ATCATTATTT
781	GCCATCCAGC TGCAGCTCTG GCCCGTGTCT	CAAAATCTCT GATGTTACAT	TGCACAAGAT
841	AAAAATATAT CATCATGAAC AATAAACTG	TCTGCTTACA TAAACAGTAA	TACAAGGGGT
901	GTTATGAGCC ATATTCAACG GGAAACGTCG	AGGCCGCGAT TAAATTCCAA	CATGGATGCT
961	GATTTATATG GGTATAAATG GGCTCGCGAT	AATGTCGGGC AATCAGGTGC	GACAATCTAT
1021	CGCTTGATG GGAAGCCCGA TGCGCCAGAG	TTGTTTCTGA AACATGGCAA	AGGTAGCGTT
1081	GCCAAATGATG TTACAGATGA GATGGTCAGA	CTAAACTGGC TGACGGAATT	TATGCCTCTT
1141	CCGACCATCA AGCATTTTAT CCGTACTCCT	GATGATGCAT GGTACTCAC	CACTGCGATC
1201	CCCGGAAAAA CAGCATTCCA GGTATTAGAA	GAATATCCTG ATTCAGGTGA	AAATATTGTT
1261	GATGCGCTGG CAGTGTTTCT GCGCCGGTTG	CATTGATTC CTGTTTGTA	TTGTCCTTTT
1321	AACAGCGATC GCGTATTTTC TCTCGCTCAG	GCGCAATCAC GAATGAATAA	CGGTTTGTTT
1381	GATGCGAGTG ATTTTGATGA CGAGCGTAAT	GGCTGGCCTG TTGAACAAGT	CTGGAAGAA
1441	ATGCATAAAC TTTTGCCATT CTCACCGGAT	TCAGTCGTCA CTCATGGTGA	TTTCTCACTT
1501	GATAACCTTA TTTTGTACGA GGGGAAATTA	ATAGGTTGTA TTGATGTTGG	ACGAGTCGGA
1561	ATCGCAGACC GATACCAGGA TCTTGCCATC	CTATGGAAC TCCCTCGGTGA	GTTTTCTCCT
1621	TCATTACAGA AACGGCTTTT TCAAAAATAT	GGTATTGATA ATCCTGATAT	GAATAAATTG
1681	CAGTTTCATT TGATGCTCGA TGAGTTTTC	TAATCAGAAT TGTTAATTG	GTTGTAAACAT
1741	TATTCAGATT GGGCCCCGTT CCACTGAGCG	TCAGACCCCG TAGAAAAGAT	CAAAGGATCT
1801	TCTTGAGATC CTTTTTTTCT GCGCGTAATC	TGCTGCTTGC AAACAAAAAA	ACCACCGCTA
1861	CCAGCGGTGG TTTGTTTGCC GGATCAAGAG	CTACCAACTC TTTTCCGAA	GGTAACGTGC
1921	TTCAGCAGAG CGCAGATACC AAATACTGTT	CTTCTAGTGT AGCCGTAGTT	AGGCCACCAC
1981	TTCAAGAACT CTGTAGCACC GCCTACATAC	CTCGCTCTGC TAATCCTGTT	ACCAGTGGCT
2041	GCTGCCAGTG GCGATAAGTC GTGTCTTACC	GGGTTGGACT CAAGACGATA	GTTACCGGAT
2101	AAGGCGCAGC GGTGCGGCTG AACGGGGGGT	TCGTGCACAC AGCCCAGCTT	GGAGCGAACC
2161	ACCTACACCG AACTGAGATA CCTACAGCGT	GAGCTATGAG AAAGCGCCAC	GCTTCCCGAA
2221	GGGAGAAAGG CGGACAGGTA TCCGGTAAGC	GGCAGGGTCG GAACAGGAGA	GCGCACGAGG
2281	GAGCTTCCAG GGGGAAACGC CTGGTATCTT	TATAGTCCTG TCGGGTTTCG	CCACCTCTGA
2341	CTTGAGCGTC GATTTTGTG ATGCTCGTCA	GGGGGGCGGA GCCTATGGAA	AAACGCCAGC
2401	AACGCGGCCT TTTTACGGTT CCTGGCCTTT	TGCTGGCCTT TTGCTCACAT	GTTCTTTCTT
2461	GCGTTATCCC CTGATTCTGT GGATAACCGT	ATTACCGCTA GCATGGATCT	CGGGGACGTC
2521	TAATACTAA GCGAGAGTAG GGAAGTCCA	GGCATCAAAT AAAACGAAAG	GCTCAGTCGG
2581	AAGACTGGGC CTTTCGTTTT ATCTGTTGTT	TGTCGGTGAA CGCTCTCCTG	AGTAGGACAA
2641	ATCCGCCGGG AGCGGATTG AACGTTGTGA	AGCAACGGCC CGGAGGGTGG	CGGGCAGGAC
2701	GCCCGCCATA AACTGCCAGG CATCAAATA	AGCAGAAGGC CATC	

FIGURE 20B

Figure 2/A: pDEST1 Native Protein Expression in E. coli

-35 Trc promoter -10 +1 RNA  
 1 atgagctgtt gacaattaat catccggctc gataatttg tggattgtg agcggataac  
 tactcgacha ctgttaatta gtagccgag catattcac accttaaac tcgcctattg  
 61 aatttcacac aggaacaga caggtatagg atcacaagtt ~~tatpdaada agctgaagta~~  
 ttaaagtgtg tcctttgtct gtccatatcc tagtgttcaa acatgtttt ~~tcgacttct~~



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## pDEST1 6464 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
216..257	Trc promoter
397..273	attR1
647..1306	CmR
1426..1510	inactivated ccdA
1648..1953	ccdB
1994..2118	attR2
2598..3503	ampR
4104..4264	ori
4504..4941	flori (f1 intergenic region)
5340..6420	lacIq

1	GTTTGACAGC	TTATCATCGA	CTGCACGGTG	CACCAATGCT	TCTGGCGTCA	GGCAGCCATC
61	GGAAGCTGTG	GTATGGCTGT	GCAGGTCGTA	AATCACTGCA	TAATTCGTGT	CGCTCAAGGC
121	GCACTCCCGT	TCTGGATAAT	GTTTTTTGCG	CCGACATCAT	AACGGTTCTG	GCAAATATTC
181	TGAAATGAGC	TGTTGACAAT	TAATCATCCG	GTCCGTATAA	TCTGTGGAAT	TGTGAGCGGG
241	ATAACAATTT	CATCGCGAGG	TACCAAGCTA	TCACAAGTTT	GTACAAAAAA	GCTGAACGAG
301	AAACGTAAAA	TGATATAAAT	ATCAATATAT	TAAATTAGAT	TTTGCAATAA	AAACAGACTA
361	CATAATACTG	TAAAAACAAA	CATATCCAGT	CACTATGGCG	GCCGCTAAGT	TGGCAGCATC
421	ACCCGACGCA	CTTTGCGCCG	AATAAATACC	TGTGACGGAA	GATCACTTCG	CAGAATAAAT
481	AAATCCTGGT	GTCCCTGTTG	ATACCGGGAA	GCCCTGGGCC	AACTTTTGGC	GAAAATGAGA
541	CGTTGATCGG	CACGTAAGAG	GTTCCAACCT	TCACCATAAT	GAAATAAGAT	CACTACCGGG
601	CGTATTTTTT	GAGTTATCGA	GATTTTCAGG	AGCTAAGGAA	GCTAAATGGG	AGAAAAAAT
661	CACTGGATAT	ACCACCGTTG	ATATATCCCA	ATGGCATCGT	AAAGAACATT	TTGAGGCATT
721	TCAGTCAGTT	GCTCAATGTA	CCTATAACCA	GACCGTTCAG	CTGGATATTA	CGGCCCTTTT
781	AAAGACCGTA	AAGAAAAATA	AGCACAAAGT	TTATCCGGCC	TTTATTCAAC	TTCTTGCCCC
841	CCTGATGAAT	GCTCATCCGG	AATTCGGTAT	GGCAATGAAA	GACGGTGAGC	TGGTGATATG
901	GGATAGTGTT	CACCCTTGTT	ACACCGTTTT	CCATGAGCAA	ACTGAAACGT	TTTCATCGCT
961	CTGGAGTGAA	TACCACGACG	ATTTCCGGCA	GTTTCTACAC	ATATATTTCG	AAGATGTGGC
1021	GTGTTACGGT	GAAAACCTGG	CCTATTTCCC	TAAAGGGTTT	ATTGAGAATA	TGTTTTTCGT
1081	CTCAGCCAAT	CCCTGGGTGA	GTTTCACCAG	TTTTGATTTA	AACGTGGCCA	ATATGGACAA
1141	CTTCTTCGCC	CCCGTTTTCA	CCATGGGCAA	ATATTATACG	CAAGGCGACA	AGGTGCTGAT
1201	GCCGCTGGCG	ATTCAGGTTT	ATCATGCCGT	CTGTGATGGC	TTCCATGTCT	GCAGAATGCT
1261	TAATGAATTA	CAACAGTACT	CCGATGAGTG	GCAGGGCGGG	GCGTAAACGC	GTGGATCCGG
1321	CTTACTAAAA	GCCAGATAAC	AGTATGCGTA	TTTGCGCGCT	GATTTTTGCG	GTATAAGAA
1381	ATATACTGAT	ATGTATACCC	GAAGTATGTC	AAAAAGAGGT	GTGCTATGAA	GCAGCGTATT
1441	ACAGTGACAG	TTGACAGCGA	CAGCTATCAG	TTGCTCAAGG	CATATATGAT	GTCAATATCT
1501	CCGGTCTGGT	AAGCACAACC	ATGCAGAATG	AAGCCCGTCG	TCTGCGTGCC	GAACGCTGGA
1561	AAGCGGAAAA	TCAGGAAGGG	ATGGCTGAGG	TCGCCCGGTT	TATTGAAATG	AACGGCTCTT
1621	TTGCTGACGA	GAACAGGGAC	TGGTGAAATG	CAGTTTAAGG	TTTACACCTA	TAAAAGAGAG
1681	AGCCGTTATC	GTCTGTTTGT	GGATGTACAG	AGTGATATTA	TTGACACGCC	CGGGCGACGG
1741	ATGGTGATCC	CCCTGGCCAG	TGCACGTCTG	CTGTCAGATA	AAGTCTCCCC	TGAACTTTAC
1801	CCGGTGGTGC	ATATCGGGGA	TGAAAGCTGG	CGCATGATGA	CCACCGATAT	GGCCAGTGTG
1861	CCGGTCTCCG	TTATCGGGGA	AGAAGTGGCT	GATCTCAGCC	ACCGCGAAAA	TGACATCAAA
1921	AACGCCATTA	ACCTGATGTT	CTGGGGAATA	TAAATGTCAG	GCTCCCTTAT	ACACAGCCAG
1981	TCTGCAGGTC	GACCATAGTG	ACTGGATATG	TTGTGTTTTA	CAGTATTATG	TAGTCTGTTT
2041	TTTATGCAAA	ATCTAATTTA	ATATATTGAT	ATTTATATCA	TTTTACGTTT	CTCGTTTCAGC
2101	TTTCTGTGAC	AAAGTGGTGA	TAGCTTGGCT	GTTTTGGCGG	ATGAGAGAAG	ATTTTCAGCC
2161	TGATACAGAT	TAAATCAGAA	CGCAGAAGCG	GTCTGATAAA	ACAGAATTTG	CCTGGCGGCA
2221	GTAGCGCGGT	GGTCCCACCT	GACCCCATGC	CGAACTCAGA	AGTGAAACGC	CGTAGCGCCG
2281	ATGGTAGTGT	GGGGTCTCCC	CATGCGAGAG	TAGGGAAGTG	CCAGGCATCA	AATAAAACGA
2341	AAGGCTCAGT	CGAAAGACTG	GGCCTTTCGT	TTTATCTGTT	GTTTGTGCGT	GAACGCTCTC
2401	CTGAGTAGGA	CAAATCCGCC	GGGAGCGGAT	TTGAACGTTG	CGAAGCAACG	GCCCGGAGGG
2461	TGGCGGGCAG	GACGCCCCGC	ATAAACTGCC	AGGCATCAAA	TTAAGCAGAA	GGCCATCCTG
2521	ACGGATGGCC	TTTTTGCGTT	TCTACAAACT	CTTTTTGTTT	ATTTTCTTAA	ATACATTCAA-

FIGURE 2/B

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2581 ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT TCAATAATAT TGAAAAAGGA  
2641 AGAGTATGAG TATTCAACAT TTCCGTGTCC CCCTTATTCC CTTTTTTGCG GCATTTTGCC  
2701 TTCCTGTTTT TGCTCACCCA GAAACGCTGG TGAAAGTAAA AGATGCTGAA GATCAGTTGG  
2761 GTGCACGAGT GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT GAGAGTTTTT  
2821 GCCCCGAAGA ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT GCGCGGTAT  
2881 TATCCCGTGT TGACGCCGGG CAAGAGCAAC TCGGTCGCCG CATACACTAT TCTCAGAATG  
2941 ACTTGGTTGA GTACTCACCA GTCACAGAAA AGCATCTTAC GGATGGCATG ACAGTAAGAG  
3001 AATTATGCAG TGCTGCCATA ACCATGAGTG ATAACACTGC GGCCAACTTA CTTCTGACAA  
3061 CGATCGGAGG ACCGAAGGAG CTAACCGCTT TTTTGCACAA CATGGGGGAT CATGTAACCT  
3121 GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG CGTGACACCA  
3181 CGATGCCTAC AGCAATGGCA ACAACGTTGC GCAAACCTATT AACTGGCGAA CTACTTACTC  
3241 TAGCTTCCCG GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA GGACCACTTC  
3301 TCGCTCGGC CCTTCCGGCT GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG  
3361 GGCTCGCGG TATCATTGCA GCACTGGGGC CAGATGGTAA GCCCTCCCGT ATCGTAGTTA  
3421 TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC GCTGAGATAG  
3481 GTGCCTCACT GATTAAGCAT TGGTAAGTGT CAGACCAAGT TTACTCATAT ATACTTTAGA  
3541 TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC  
3601 TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA  
3661 AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAACAA  
3721 AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC  
3781 CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAATAC TGTCTTCTA GTGTAGCCGT  
3841 AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC  
3901 TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC  
3961 GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGTTTCGTGC ACACAGCCCA  
4021 GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG  
4081 CCACGCTTCC CGAAGGGAGA AAGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG  
4141 GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCCGGT  
4201 TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CCGAGCCTAT  
4261 GAAAAAACCG CAGCAACGCG GCCTTTTTAC GGTTCCTGGC CTTTGTCTGG CTTTGTCTC  
4321 ACATGTTCTT TCCTGCGTTA TCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT  
4381 GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG  
4441 CGGAAGAGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA  
4501 TAATTTTGTT AAAATTTCGG TTAATTTTTT GTTAAATCAG CTCATTTTTT AACCAATAGG  
4561 CCGAAATCGG CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG TTGAGTGTG  
4621 TTCCAGTTTG GAACAAGAGT CCACTATTAA AGAACGTGGA CTCCAACGTC AAAGGGCGAA  
4681 AAACCGTCTA TCAGGGCGAT GGCCCACTAC GTGAACCATC ACCCTAATCA AGTTTTTTGG  
4741 GGTCGAGGTG CCGTAAAGCA CTAAATCGGA ACCCTAAAGG GAGCCCCCGA TTTAGAGCTT  
4801 GACGGGGAAA GCCGGCGAAG GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG  
4861 CTAGGGCGCT GGCAAGTGTG GCGGTCACGC TGCGCGTAAC CACCACACCC GCCGCGTTA  
4921 ATGCGCCGCT ACAGGGCGCG TCCATTGCGC ATTGAGGCTG CTATGGTGCA CTCTCAGTAC  
4981 AATCTGCTCT GATGCCGCAT AGTTAAGCCA GTACAGTCA CGTAGCGATA TCGGAGTGTA  
5041 TACTACTCCG TATCGCTACG TGACTGGGTC ATGGCTGCGC CCCGACACCC GCCAACACCC  
5101 GCTGACGCGC CCTGACGGGC TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC  
5161 GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGAGGCAG  
5221 CAGATCAATT CGCGCGCGAA GGCGAAGCGG CATGCATTTA CGTTGACACC ATCGAATGGT  
5281 GCAAAACCTT TCGCGGTATG GCATGATAGC GCCCGGAAGA GAGTCAATTC AGGGTGGTGA  
5341 ATGTGAAACC AGTAACGTTA TACGATGTCG CAGAGTATGC CGGTGTCTCT TATCAGACCG  
5401 TTTCCCGCGT GGTGAACCAG GCCAGCCACG TTTCTGCGAA AACGCGGGAA AAAGTGGAAAG  
5461 CGCGATGGC GGAGCTGAAT TACATTCCCA ACCGCGTGGC ACAACAACCT GCGGGCAAC  
5521 AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT GCACGCGCCG TCGCAAATTG  
5581 TCGCGGCGAT TAAATCTCGC GCCGATCAAC TGGGTGCCAG CGTGGTGGTG TCGATGGTAG  
5641 AACGAAGCGG CGTCGAAGCC TGTAAAGCGG CCGTGACAAA TCTTCTCGCG CAACGCGTCA  
5701 GTGGGCTGAT CATTAACTAT CCGCTGGATG ACCAGGATGC CATTGCTGTG GAAGCTGCCT  
5761 GCACTAATGT TCCGGCGTTA TTTCTTGATG TCTCTGACCA GACACCCATC AACAGTATTA  
5821 TTTTCTCCCA TGAAGACGGT ACGCGACTGG GCGTGAGCA TCTGGTCGCA TTGGGTCAAC  
5881 AGCAAATCGC GCTGTTAGCG GGCCCAATTAA GTTCTGTCTC GCGCGCTCTG CGTCTGGCTG  
5941 GCTGGCATAA ATATCTCACT CGCAATCAAA TTCAGCCGAT AGCGGAACGG GAAGGCGACT  
6001 GGAGTGCCAT GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA-

FIGURE 21C

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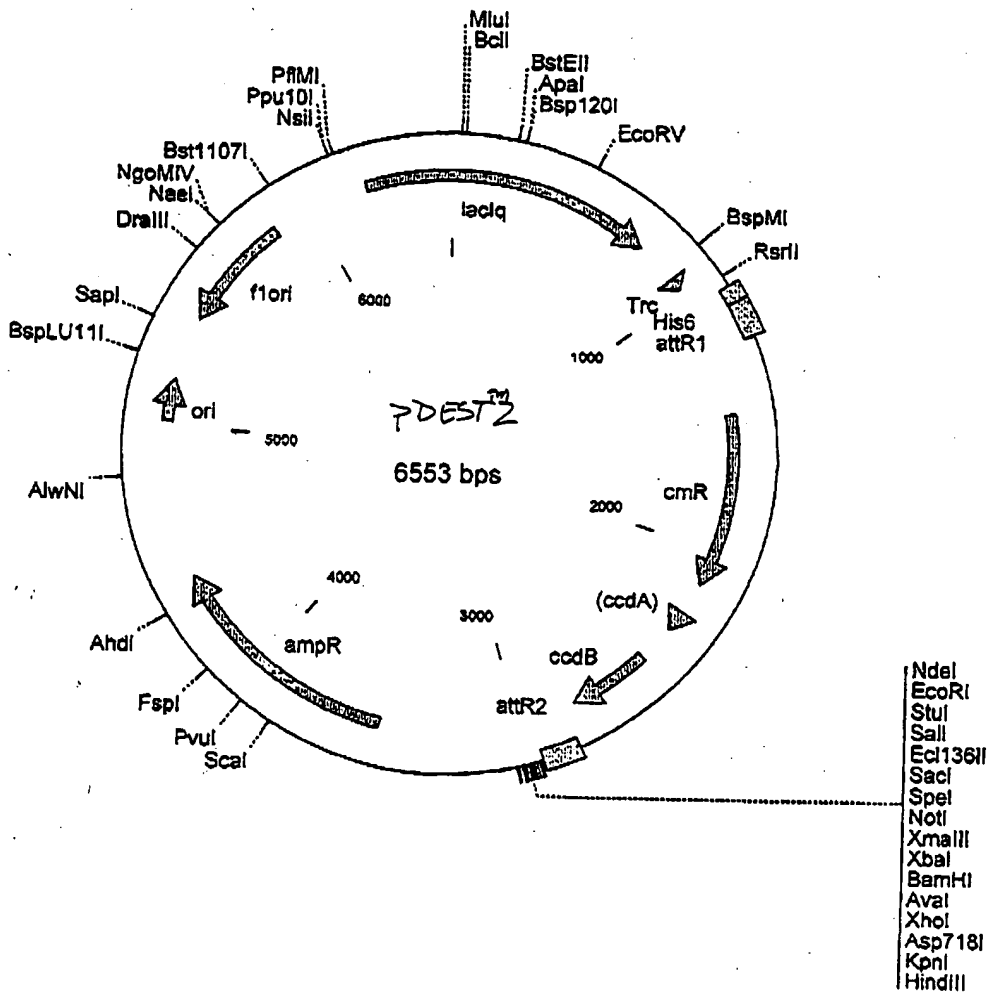
6061 CTGCGATGCT GGTGCCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC ATTACCGAGT  
6121 CCGGGCTGCG CGTTGGTGCG GATATCTCGG TAGTGGGATA CGACGATACC GAAGACAGCT  
6181 CATGTTATAT CCCGCCGTTA ACCACCATCA AACAGGATTT TCGCCTGCTG GGGCAAACCA  
6241 GCGTGGACCG CTTGCTGCAA CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC  
6301 CCGTCTCACT GGTGAAAAGA AAAACCACCC TGGCACCCAA TACGCAAACC GCCTCTCCCC  
6361 GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC  
6421 AGTGAGCGCA ACGCAATTAA TGTGAGTTAG CGCGAATTGA TCTG

FIGURE 21D

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Figure 22A:  $\text{pDEST2}$ His6 fusions in *E. coli*

970 aat att ctg aaa tga gct <sup>-35</sup> gtt gac aat tad tca tcc ggt ccg <sup>-10</sup> tat aat ctg  
 tta taa gac ttt act cga ~~aaa ctg tca~~ att agt agg cca ggc ~~ata tta~~ gac  
 1021 tgg <sup>RNA</sup> aat tgt gag cgg ata aca att tca cac agg aaa cag acc <sup>Met Ser Tyr</sup> atg tgg tac  
 acc tta aca ctc gcc tat tgt taa agt gtc tcc ttt gtc tgg tac agc atg  
 1072 <sup>Tyr His His His His His His</sup> tac cat cac cat cat cat cat <sup>Glu Ile</sup> ggt att ~~tca agt tgg~~ <sup>Trp Ser Pst</sup> ~~cat~~ <sup>attR1</sup> ~~aaa gct gaa~~  
 atg gta gtg gta gtg gta gtg ccg tag ~~tgt tca aac atg ttt~~ ~~ctt cga ctt~~





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## pDEST2 6553 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
912..962		Trc
1223..1009		attR1
1473..2132		CmR
2252..2336		inactivated ccdA
2474..2779		ccdB
2820..2944		attR2
3509..4414		ampR
5015..5175		ori
5415..5852		flori (f1 intergenic region)
6225..752		lacIq

1	GGCGGTGCAC	AATCTTCTCG	CGCAACGCGT	CAGTGGGCTG	ATCATTAACT	ATCCGCTGGA
61	TGACCAGGAT	GCCATTGCTG	TGGAAGCTGC	CTGCACTAAT	GTTCCGGCGT	TATTTCTTGA
121	TGTCTCTGAC	CAGACACCCA	TCAACAGTAT	TATTTTCTCC	CATGAAGACG	GTACGCGACT
181	GGGCGTGGAG	CATCTGGTGC	CATTGGGTCA	CCAGCAAATC	GCGCTGTTAG	CGGGCCCAAT
241	AAGTTCTGTC	TCGGCGCGTC	TGCGTCTGGC	TGGCTGGCAT	AAATATCTCA	CTCGCAATCA
301	AATTCAGCCG	ATAGCGGAAC	GGGAAGGCGA	CTGGAGTGCC	ATGTCCGGTT	TTCAACAAAC
361	CATGCAAATG	CTGAATGAGG	GCATCGTTCC	CACTGCGATG	CTGGTTGCCA	ACGATCAGAT
421	GGCGCTGGGC	GCAATGCGCG	CCATTACCGA	GTCCGGGCTG	CGCGTTGGTG	CGGATATCTC
481	GGTAGTGGGA	TACGACGATA	CCGAAGACAG	CTCATGTTAT	ATCCCGCCGT	CAACCACCAT
541	CAAACAGGAT	TTTCGCCTGC	TGGGGCAAAC	CAGCGTGGAC	CGCTTGCTGC	AACTCTCTCA
601	GGGCCAGGCG	GTGAAGGGCA	ATCAGCTGTT	GCCCGTCTCA	CTGGTGAAAA	GA AAAACCAC
661	CCTGGCACCC	AATACGCAAA	CCGCCTCTCC	CCGCGCGTTG	GCCGATTCAT	TAATGCAGCT
721	GGCACGACAG	GTTTCCCGAC	TGGAAGCGG	GCACTGAGCG	CAACGCAATT	AATGTGAGTT
781	AGCGCGAATT	GATCTGGTTT	GACAGCTTAT	CATCGACTGC	ACGGTGCACC	AATGCTTCTG
841	GCGTCAGGCA	GCCATCGGAA	GCTGTGGTAT	GGCTGTGCAG	GTCTGTAATC	ACTGCATAAT
901	TCGTGTCGCT	CAAGGCGCAC	TCCCGTCTG	GATAATGTTT	TTTGCGCCGA	CATCATAACG
961	GTTCTGGCAA	ATATTCTGAA	ATGAGCTGTT	GACAATTAAT	CATCCGGTCC	GTATAATCTG
1021	TGGAATTGTG	AGCGGATAAC	AATTTACAC	AGGAAACAGA	CCATGTCGTA	CTACCATCAC
1081	CATCACCATC	ACGGCATCAC	AAGTTTGATC	AAAAAAGCTG	AACGAGAAAC	GTAAATGAT
1141	ATAAATATCA	ATATATTAAA	TTAGATTTTG	CATAAAAAAC	AGACTACATA	ATACTGTAAA
1201	ACACAACATA	TCCAGTCACT	ATGGCGGCCG	CTAAGTTGGC	AGCATCACCC	GACGCACTTT
1261	GCGCCGAATA	AATACCTGTG	ACGGAAGATC	ACTTCGCAGA	ATAAATAAAT	CCTGGTGTC
1321	CTGTTGATAC	CGGGAAGCCC	TGGGCCAAT	TTTGCGGAAA	ATGAGACGTT	GATCGGCACG
1381	TAAGAGGTTT	CAACTTTCAC	CATAATGAAA	TAAGATCACT	ACCGGGCGTA	TTTTTTGAGT
1441	TATCGAGATT	TTCAGGAGCT	AAGGAAGCTA	AAATGGAGAA	AAAAATCACT	GGATATACCA
1501	CCGTTGATAT	ATCCCAATGG	CATCGTAAAG	AACATTTTGA	GGCATTTTCA	TCAGTTGCTC
1561	AATGTACCTA	TAACCAGACC	GTTTCTGCTG	ATATTACGGC	CTTTTAAAG	ACCGTAAAGA
1621	AAAATAAGCA	CAAGTTTAT	CCGGCCTTTA	TTTACATTCT	TGCCCCGCTG	ATGAATGCTC
1681	ATCCGGAATT	CCGTATGGCA	ATGAAAGACG	GTGAGCTGGT	GATATGGGAT	AGTGTTCACC
1741	CTTGTTACAC	CGTTTTCCAT	GAGCAAACCTG	AAACGTTTTC	ATCGCTCTGG	AGTGAATACC
1801	ACGACGATTT	CCGGCAGTTT	CTACACATAT	ATTTCGCAAGA	TGTGGCGTGT	TACGGTGAAA
1861	ACCTGGCCTA	TTTCCCTAAA	GGGTTTATTG	AGAATATGTT	TTTCGTCTCA	GCCAATCCCT
1921	GGGTGAGTTT	CACCAAGTTT	GATTTAAACG	TGGCCAATAT	GGACAACTTC	TTCCGCCCCG
1981	TTTTCAACAT	GGGCAAATAT	TATACGCAAG	GCGACAAGGT	GCTGATGCCG	CTGGCGATTTC
2041	AGGTTTCATCA	TGCCGTCTGT	GATGGCTTCC	ATGTCGGCAG	AATGCTTAAT	GAATTACAAC
2101	AGTACTGCGA	TGAGTGGCAG	GGCGGGGCGT	AAACGCGTGG	ATCCGGCTTA	CTAAAAGCCA
2161	GATAACAGTA	TGCGTATTGT	GCGCTGATT	TTTGCGGTAT	AAGAATATAT	ACTGATATGT
2221	ATACCCGAAG	TATGTCAAAA	AGAGGTGTGC	TATGAAGCAG	CGTATTACAG	TGACAGTTGA
2281	CAGCGACAGC	TATCAGTTGC	TCAAGGCATA	TATGATGTCA	ATATCTCCGG	TCTGGTAAGC
2341	ACAACCATGC	AGAATGAAGC	CCGTCGTCTG	CGTGCCGAAC	GCTGGAAAGC	GGAAAATCAG
2401	GAAGGGATGG	CTGAGGTCGC	CCGGTTTATT	GAAATGAACG	GCTCTTTTGC	TGACGAGAAC
2461	AGGGACTGGT	GAAATGCAGT	TTAAGGTTTA	CACCTATAAA	AGAGAGAGCC	GTTATCGTCT
2521	GTTTGTGGAT	GTACAGAGTG	ATATTATTGA	CACGCCCGGG	CGACGGATGG	TGATCCCCCT-

FIGURE 22B

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2581 GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA CTTTACCCGG TGGTGCATAT  
2641 CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC AGTGTGCCGG TCTCCGTTAT  
2701 CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC ATCAAAAACG CCATTAACTT  
2761 GATGTTCTGG GGAATATAAA TGTCAGGCTC CTTTATACAC AGCCAGTCTG CAGGTCGACC  
2821 ATAGTGACTG GATATGTTGT GTTTTACAGT ATTATGTAGT CTGTTTTTTA TGCAAAATCT  
2881 AATTTAATAT ATTGATATTT ATATCATTTT ACGTTTCTCG TTCAGCTTTC TTGTACAAAG  
2941 TGGTGATGCC CATATGGGAA TTCAAAGGCC TACGTGCGAC AGCTCACTAG TCGCGGCCGC  
3001 TTCTAGAGGA TCCCTCGAGG CATGCGGTAC CAAGCTTGGC TGTTTTGGCG GATGAGAGAA  
3061 GATTTTCAGC CTGATACAGA TTAAATCAGA ACGCAGAAGC GGTCTGATAA AACAGAAATTT  
3121 GCCTGGCGGC AGTAGCGCGG TGSTCCACCC TGACCCCATG CCGAACTCAG AAGTGAAACG  
3181 CCGTAGCGCC GATGGTAGTG TGGGGTCTCC CCATGCGAGA GTAGGGAAC T GCCAGGCATC  
3241 AAATAAAACG AAAGGCTCAG TCGAAAGACT GGGCCTTTCG TTTTATCTGT TGTTTGTGG  
3301 TGAACGCTCT CCTGAGTAGG ACAAATCCGC CGGGAGCGGA TTGAACGTT CGGAAGCAAC  
3361 GGCCCGGAGG GTGGCGGGCA GGACGCCCGC CATAAACTGC CAGGCATCAA ATTAAGCAGA  
3421 AGGCCATCCT GACGGATGGC CTTTTGCGT TTCTACAAAC TCTTTTTGTT TATTTTTCTA  
3481 AATACATTCA AATATGTATC CGCTCATAG ACATAAACC TGATAAATGC TTCAATAATA  
3541 TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CCTTTTTTGC  
3601 GGCATTTTGC CTTCTGTTT TTGCTCACC AGAAACGCTG GTGAAAGTAA AAGATGCTGA  
3661 AGATCAGTTG GGTGCACGAG TGGGTACAT CGAAGTGGAT CTCAACAGCG GTAAGATCCT  
3721 TGAGAGTTTT CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG  
3781 TGGCGCGGTA TTATCCCGTG TTGACGCCGG GCAAGAGCAA CTCGGTCGCC GCATACACTA  
3841 TTCTCAGAA GACTTGTTG AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT  
3901 GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACTT  
3961 ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCGCT TTTTTCACA ACATGGGGGA  
4021 TCATGTAAC TCGCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA  
4081 GCGTGACACC ACGATGCCTA CAGCAATGGC AACACGTTG CGCAAATAT TAACTGGCGA  
4141 ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC  
4201 AGGACCACTT CTGCGCTCGG CCTTCCGGC TGGCTGTTT ATTGCTGATA AATCTGGAGC  
4261 CGGTGAGCGT GGGTCTCGCG GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCCTCCCG  
4321 TATCGTAGTT ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT  
4381 CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA  
4441 TATACTTTAG ATTGATTAA AACTTCATT TTAATTAAA AGGATCTAGG TGAAGATCCT  
4501 TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTCCACT GAGCGTCAGA  
4561 CCCCGTAGAA AAGATCAAAG GATCTTCTG AGATCCTTTT TTTCTGCGG TAATCTGCTG  
4621 CTTGCAAAACA AAAAAACCA CGCTACCAG GGTGTTTGT TTGCCGATC AAGAGCTACC  
4681 AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT  
4741 AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCTA CATACCTCGC  
4801 TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGT  
4861 GGAATCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTG GGTGAACGG GGGGTTCTG  
4921 CACACAGCCC AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT  
4981 ATGAGAAAGC GCCACGCTT CCGAAGGGAG AAAGCGGAC AGGTATCCG TAAGCGGCAG  
5041 GGTCCGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG  
5101 TCCTGTGCGG TTTGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG  
5161 GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCTTGG CCTTTTGTCTG  
5221 GCCTTTTGCT CACATGTTCT TTCTGCGTT ATCCCTGAT TCTGTGGATA ACCGTATTAC  
5281 CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT  
5341 GAGCGAGGAA GCGGAAGAGC GCCTGATGCG GTATTTTCTC CTTACGCATC TGTGCGGTAT  
5401 TTGACACCGC ATAATTTGT TAAATTTCG GTTAAATTTT TGTAAATCA GCTCATTTT  
5461 TAACCAATAG GCCGAAATCG GCAAAATCCC TTATAAATCA AAAGAATAGA CCGAGATAGG  
5521 GTTGAGTGTT GTTCCAGTTT GGAACAAGAG TCCACTATTA AAGAACGTGG ACTCCAACGT  
5581 CAAAGGGCGA AAAACCGTCT ATCAGGGCGA TGGCCCACTA CGTGAACCAT CACCCTAATC  
5641 AAGTTTTTTG GGGTCGAGGT GCCGTAAAGC ACTAAATCGG AACCCCTAAG GGAGCCCCCG  
5701 ATTTAGAGCT TGACGGGAA AGCCGGCGAA CGTGGCGAGA AAGGAAGGGA AGAAAGCGAA  
5761 AGGAGCGGC GCTAGGGCGC TGGCAAGTGT AGCGGTACG CTGCGCGTAA CCACCACACC  
5821 CGCCGCGCTT AATGCGCGC TACAGGGCGC GTCCCATTCG CCATTCAAGC TGCTATGGTG  
5881 CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAGTATACAC TCCGCTATCG  
5941 CTACGTGACT GGTCTATGGC TGCGCCCCGA CACCCGCCAA CACCCGCTGA CGCGCCCTGA  
6001 CGGGCTTGTC TGCTCCCGGC ATCCGCTTAC AGACAAGCTG TGACCGTCTC CGGGAGCTGC-

FIGURE 22C

6061 ATGTGTCAGA GGTTTTCACC GTCATCACCG AAACGCGCGA GGCAGCAGAT CAATTCGCGC  
6121 GCGAAGGCGA AGCGGCATGC ATTTACGTTG ACACCATCGA ATGGTGCAAA ACCTTTCGCG  
6181 GTATGGCATG ATAGCGCCCG GAAGAGAGTC AATTCAGGGT GGTGAATGTG AAACCAGTAA  
6241 CGTTATACGA TGTCGCAGAG TATGCCGGTG TCTCTTATCA GACCGTTTCC CGCGTGGTGA  
6301 ACCAGGCCAG CCACGTTTCT GCGAAAACGC GGGAAAAAGT GGAAGCGGCG ATGGCGGAGC  
6361 TGAATTACAT TCCCAACCGC GTGGCACAAC AACTGGCGGG CAAACAGTCG TTGCTGATTG  
6421 GCGTTGCCAC CTCCAGTCTG GCCCTGCACG CGCCGTCGCA AATTGTCGCG GCGATTAAAT  
6481 CTCGCGCCGA TCAACTGGGT GCCAGCGTGG TGGTGTGCGT GGTAGAACGA AGCGGCGTCG  
6541 AAGCCTGTAA AGC

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Figure 23A: PDEST3

GST fusions in E. coli

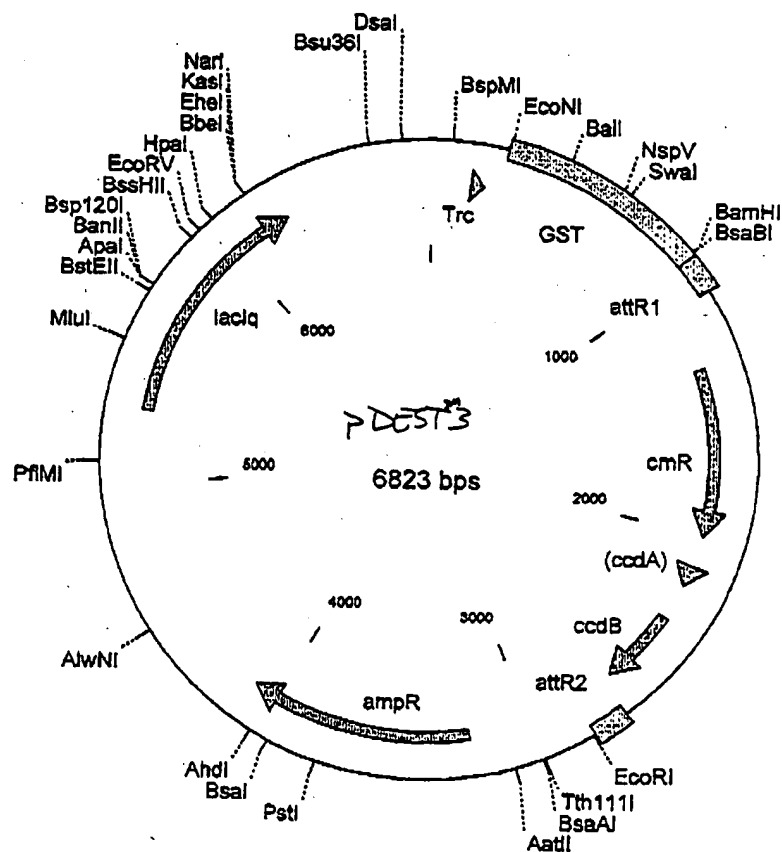
154 cgg ttc tgg caa ata ttc tga aat gag ctg <sup>-35</sup> ttg aca att aat cat cgg ctc  
 gcc aag acc gtt tat aag act tta ctc gac aac tgt taa tta gta gcc gag

205 <sup>-10</sup> gta taa agt gtg gaa ttg tga gcg gat aac aat ttc aca cag gaa aca gta  
 cat att aca cac ctt aac act cgc cta ttg tta aag tgt gtc ctt tgt cat

256 <sup>M S P I L</sup> ttc atg tcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg caa ccc  
 aag tac agg gga tat gat cca ata acc ttt taa ttc ccg gaa cac gtt ggg

919 <sup>" GST</sup> ctg gtt ccg cgt gga tct cgt cgt gca tct gtt gga tcc cca tca <sup>T S</sup> aca agt  
 gac caa ggc gca cct aga gca gca cgt aga caa cct agg ggt agt tgt tca

970 <sup>H Y K K</sup> tgg ttc taa gaa gct gaa cga gaa acg taa aat gat ata aat gct aat ata  
 aac atg ttt tct cga cct gct cct tgc att tta cta tat tta tag tta tat



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## pDEST3 6823 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
150..200		Trc
1087..963		attR1
1337..1996		CmR
2116..2200		inactivated ccdA
2338..2643		ccdB
2684..2808		attR2
3231..4091		ampR
5295..6254		lacIq

1	ACGTTATCGA	CTGCACGGTG	CACCAATGCT	TCTGGCGTCA	GGCAGCCATC	GGAAGCTGTG
61	GTATGGCTGT	GCAGGTCGTA	AATCACTGCA	TAATTCGTGT	CGCTCAAGGC	GCACTCCCGT
121	TCTGGATAAT	GTTTTTTGCG	CCGACATCAT	AACGGTTCTG	GCAAATATTC	TGAAATGAGC
181	TGTTGACAAT	TAATCATCGG	CTCGTATAAT	GTGTGGAATT	GTGAGCGGAT	AACAATTTCa
241	CACAGGAAAC	AGTATTCATG	TCCCCTATAC	TAGGTTATTG	GAAAATTAAG	GGCCTTGTC
301	AACCCACTCG	ACTTCTTTTG	GAATATCTTG	AAGAAAAATA	TGAAGAGCAT	TTGTATGAGC
361	GCGATGAAGG	TGATAAATGG	CGAAACAAAA	AGTTTGAATT	GGGTTTGGAG	TTTCCCAATC
421	TTCTTTATTA	TATTGATGGT	GATGTTAAAT	TAACACAGTC	TATGGCCATC	ATACGTTATA
481	TAGCTGACAA	GCACAACATG	TTGGGTGGTT	GTCCAAAAGA	GCGTGACAGG	ATTTCAATGC
541	TTGAAGGAGC	GGTTTTGGAT	ATTAGATACG	GTGTTTCGAG	AATTGCATAT	AGTAAAGACT
601	TTGAAACTCT	CAAAGTTGAT	TTTCTTAGCA	AGCTACCTGA	AATGCTGAAA	ATGTTCCAAG
661	ATCGTTTATG	TCATAAAACA	TATTTAAATG	GTGATCATGT	AACCCATCCT	GACTTCATGT
721	TGTATGACGC	TCTTGATGTT	GTTTTATACA	TGGACCCAAT	GTGCCTGGAT	GCGTTCCCAA
781	AATTAGTTTG	TTTTAAAAAA	CGTATTGAAG	CTATCCCAAC	AATTGATAAG	TACTTGAAT
841	CCAGCAAGTA	TATAGCATGG	CCTTTCGAGG	GCTGGCAAGC	CACGTTTGGT	GGTGGCGACC
901	ATCCTCCAAA	ATCGGATCTG	GTTCCGCGTG	GATCTCGTCG	TGCATCTGTT	GGATCCCAT
961	CAACAAGTTT	GTACAAAAAA	GCTGAACGAG	AAACGTAAAA	TGATATAAAT	ATCAATATAT
1021	TAAATTAGAT	TTTGCATAAA	AAACAGACTA	CATAACTCTG	TAAAACACAA	CATATCCAGT
1081	CACTATGGCG	GCCGCTAAGT	TGGCAGCATC	ACCCGACGCA	CTTTGCGCCG	AATAAATACC
1141	TGTGACGGAA	GATCACTTCG	CAGAATAAAT	AAATCCTGGT	GTCCCTGTTG	ATACCGGGAA
1201	GCCCTGGGCC	AACTTTTGGC	GAAAATGAGA	CGTTGATCGG	CACGTAAGAG	GTTCCAACCT
1261	TCACCATAAT	GAAATAAGAT	CACTACCGGG	CGTATTTTTT	GAGTTATCGA	GATTTTCAGG
1321	AGCTAAGGAA	GCTAAAATGG	AGAAAAAAT	CACTGGATAT	ACCACCGTTG	ATATATCCCA
1381	ATGGCATCGT	AAAGAACATT	TTGAGGCATT	TCAGTCAGTT	GCTCAATGTA	CCTATAACCA
1441	GACCGTTTCG	CTGGATATTA	CGGCCTTTTT	AAAGACCGTA	AAGAAAAATA	AGCACAAGTT
1501	TTATCCGGCC	TTTATTCACA	TTCTTGCCCG	CCTGATGAAT	GCTCATCCGG	AATTCCGTAT
1561	GGCAATGAAA	GACGGTGAGC	TGGTGATATG	GGATAGTGTT	CACCCTTGTT	ACACCGTTTT
1621	CCATGAGCAA	ACTGAAACGT	TTTCATCGCT	CTGGAGTGAA	TACCACGACG	ATTTCCGGCA
1681	GTTTCTACAC	ATATATTTCG	AAGATGTGGC	GTGTTACGGT	GAAAACCTGG	CCTATTTCCC
1741	TAAAGGGTTT	ATTGAGAATA	TGTTTTTCGT	CTCAGCCAAT	CCCTGGGTGA	GTTTCACCAG
1801	TTTGTATTTA	AACGTGGCCA	ATATGGACAA	CTTCTTCGCC	CCCGTTTTCA	CCATGGGCAA
1861	ATATTATACG	CAAGGCGACA	AGGTGCTGAT	GCCGCTGGCG	ATTCAGGTTT	ATCATGCCGT
1921	CTGTGATGGC	TTCCATGTCG	GCAGAATGCT	TAATGAATTA	CAACAGTACT	GCGATGAGTG
1981	GCAGGGCGGG	GCGTAAAGAT	CTGGATCCGG	CTTACTAAAA	GCCAGATAAC	AGTATGCGTA
2041	TTTGCGCGCT	GATTTTTTCG	GTATAAGAAT	ATATACTGAT	ATGTATACCC	GAAGTATGTC
2101	AAAAAGAGGT	GTGCTATGAA	GCAGCGTATT	ACAGTGACAG	TTGACAGCGA	CAGCTATCAG
2161	TTGCTCAAGG	CATATATGAT	GTCAATATCT	CCGGTCTGGT	AAGCACAACC	ATGCAGAATG
2221	AAGCCCCGTC	TCTGCGTGCC	GAACGCTGGA	AAGCGGAAAA	TCAGGAAGGG	ATGGCTGAGG
2281	TCGCCCCGTT	TATTGAAATG	AACGGCTCTT	TTGCTGACGA	GAACAGGGAC	TGGTGAAATG
2341	CAGTTTAAGG	TTTACACCTA	TAAAAGAGAG	AGCCGTTATC	GTCTGTTTGT	GGATGTACAG
2401	AGTGATATTA	TTGACACGCC	CGGGCGACGG	ATGGTGATCC	CCCTGGCCAG	TGCACGCTCG
2461	CTGTCAGATA	AAGTCTCCCG	TGAACCTTAC	CCGGTGGTGC	ATATCGGGGA	TGAAAGCTGG
2521	CGCATGATGA	CCACCGATAT	GGCCAGTGTG	CCGGTCTCCG	TTATCGGGGA	AGAAGTGCGT
2581	GATCTCAGCC	ACCGCGAAAA	TGACATCAAA	AACGCCATTA	ACCTGATGTT	CTGGGGAATA
2641	TAAATGTCAG	GCTCCCTTAT	ACACAGCCAG	TCTGCAGGTC	GACCATAGTG	ACTGGATATG

FIGURE 23B

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2701 TTGTGTTTTA CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA ATATATTGAT  
2761 ATTTATATCA TTTTACGTTT CTCGTTACAGC TTTCTTGTAAC AAAGTGGTTG ATGGGAATTC  
2821 ATCGTGACTG ACTGACGATC TGCCTCGCGC GTTTCGGTGA TGACGGTGAA AACCTCTGAC  
2881 ACATGCAGCT CCCGGAGACG GTCACAGCTT GTCTGTAAGC GGATGCCGGG AGCAGACAAG  
2941 CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG GGTGTCGGGG CGCAGCCATG ACCCAGTCAC  
3001 GTAGCGATAG CGGAGTGTAT AATTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT  
3061 TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCCGGGAA  
3121 ATGTGCGCGG AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA  
3181 TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC  
3241 AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC  
3301 ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT  
3361 ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT  
3421 TTCCAATGAT GAGCACTTTT AAAGTTCCTG TATGTGGCGC GGTATTATCC CGTGTGACG  
3481 CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT  
3541 CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGTG  
3601 CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA  
3661 AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG  
3721 AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA  
3781 TGGCAACAAC GTTGGCAGAA CTATTAACATG GCGAACTACT TACTCTAGCT TCCCGGCAAC  
3841 AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC  
3901 CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA  
3961 TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA  
4021 GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA  
4081 AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAAGACTTC  
4141 ATTTTTAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAATCC  
4201 CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT  
4261 CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC  
4321 CAGCGGTGGT TTGTTTGGCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAAGTGGCT  
4381 TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT  
4441 TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG  
4501 CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGAATC AAGACGATAG TTACCGGATA  
4561 AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACACA GCCCAGCTTG GAGCGAACGA  
4621 CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG  
4681 GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCAGGAGG  
4741 AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCTGTG CGGGTTTCGC CACTCTGAC  
4801 TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACCGCAGCA  
4861 ACGCGGCCCT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG  
4921 CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC  
4981 GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCTGA  
5041 TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATAAAT TCCGACACCA  
5101 TCGAATGGTG CAAAACCTTT CGCGGTATGG CATGATAGCG CCCGGAAGAG AGTCAATTCA  
5161 GGGTGGTGAA TGTGAAACCA GTAACGTTAT ACGATGTCGC AGAGTATGCC GGTGTCTCTT  
5221 ATCAGACCGT TTCCCGCGTG GTGAACCAAG CCAGCCACGT TTCTGCGAAA ACGCGGGAAA  
5281 AAGTGGAAGC GGCGATGGCG GAGCTGAATT ACATTCCCAA CCGCGTGGCA CAACAACTGG  
5341 CGGGCAAACA GTCGTTGCTG ATTGGCGTTG CCACCTCCAG TCTGGCCCTG CACGCGCCGT  
5401 CGCAAATTGT CGCGGCGATT AAATCTCGCG CCGATCAACT GGGTGCCAGC GTGGTGGTGT  
5461 CGATGGTAGA ACGAAGCGGC GTCGAAGCCT GTAAAGCGGC GGTGCACAAAT CTTCTCGCGC  
5521 AACCGGTCAG TGGGCTGATC ATTAACATATC CGCTGGATGA CCAGGATGCC ATTGTCTGGG  
5581 AAGCTGCCTG CACTAATGTT CCGGCGTTAT TTCTTGATGT CTCTGACCAG ACACCCATCA  
5641 ACAGTATTAT TTTCTCCCAT GAAGACGGTA CGCGACTGGG CGTGGAGCAT CTGGTTCGAT  
5701 TGGGTCACCA GCAAATCGCG CTGTTAGCGG GCCCATTAAG TTCTGTCTCG GCGCGTCTGC  
5761 GTCTGGCTGG CTGGCATAAA TATCTCACTC GCAATCAAAT TCAGCCGATA GCGGAACGGG  
5821 AAGGCGACTG GAGTGCCATG TCCGGTTTTT AACAAACCAT GCAAATGCTG AATGAGGGCA  
5881 TCGTTCCAC TCGATGCTG GTTGCCAACG ATCAGATGGC GCTGGGCGCA ATGCGCGCCA  
5941 TTACCAGATC CGGGCTGCGC GTTGGTGCGG ATATCTCGGT AGTGGGATAC GACGATACCG  
6001 AAGACAGCTC ATGTTATATC CCGCGGTTAA CCACCATCAA ACAGGATTTT CGCCTGCTGG  
6061 GGCAAACCAAG CGTGGACCGG TTGCTGCAAC TCTCTCAGGG CCAGGCGGTG AAGGGCAATC  
6121 AGCTGTTGCC CGTCTCACTG GTGAAAAGAA AAACCACCCT GGCGCCCAAT ACGCAAACCG-

FIGURE 23C

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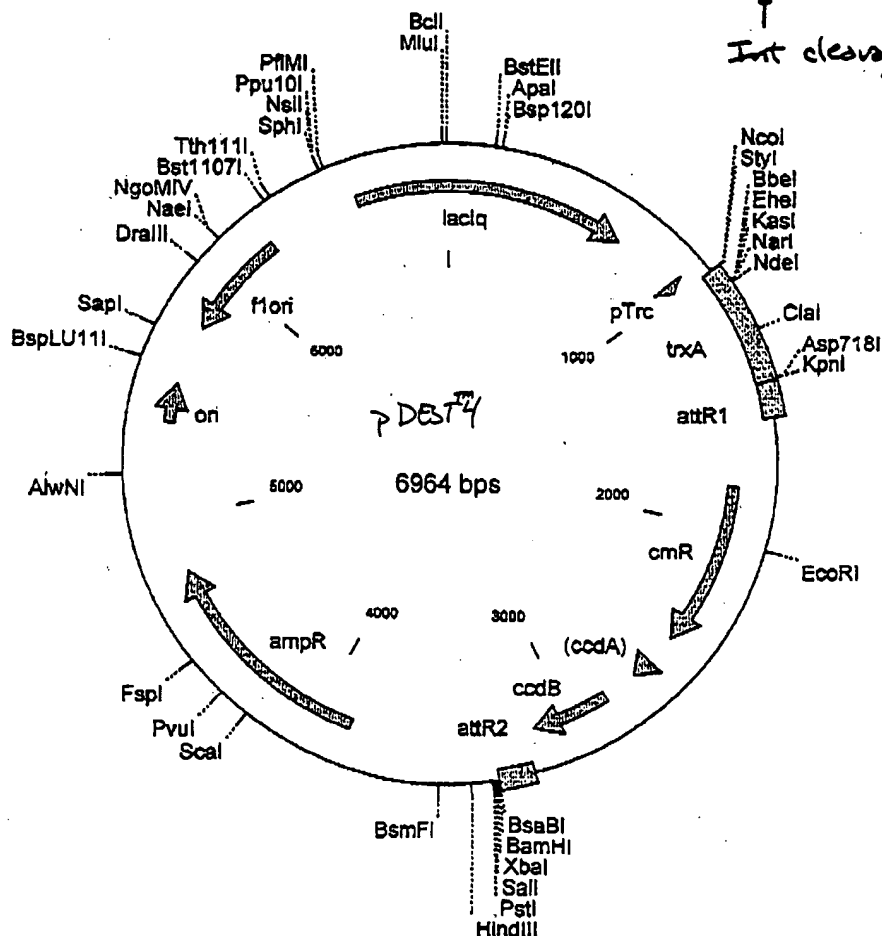
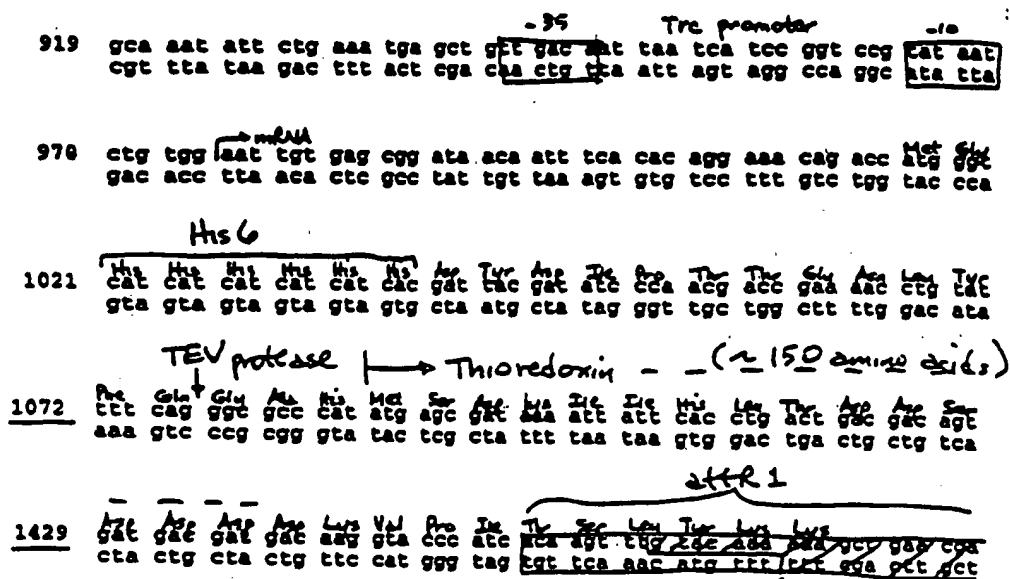
6181 CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC ACGACAGGTT TCCCGACTGG  
6241 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC TCACTCATTA GGCACCCAG  
6301 GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT  
6361 CACACAGGAA ACAGCTATGA CCATGATTAC GGATTCACTG GCCGTCGTTT TACAACGTCG  
6421 TGA CTGGGAA AACCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC  
6481 CAGCTGGCGT AATAGCGAAG AGGCCCCGAC CGATCGCCCT TCCCAACAGT TGCGCAGCCT  
6541 GAATGGCGAA TGGCGCTTTG CCTGGTTTCC GGCACCAGAA GCGGTGCCGG AAAGCTGGCT  
6601 GGAGTGGGAT CTTCTGAGG CCGATACTGT CGTCGTCCCC TCAAACCTGGC AGATGCACGG  
6661 TTACGATGCG CCCATCTACA CCAACGTAAC CTATCCCATT ACGGTCAATC CGCCGTTTGT  
6721 TCCCACGGAG AATCCGACGG GTTGTTACTC GCTCACATTT AATGTTGATG AAAGCTGGCT  
6781 ACAGGAAGGC CAGACGCGAA TTATTTTGA TGGCGTTGGA ATT

FIGURE 23D

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Figure 24A: pDEST4

## His6-thioredoxin fusions in E. coli





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## pDEST4 6964 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
964..1003		Trc
1577..1453		attR1
1827..2486		CmR
2606..2690		inactivated ccdA
2828..3133		ccdB
3174..3298		attR2
3872..4777		ampR
5378..5538		ori
5778..6215		flori (f1 intergenic region)
6587..704		lacIq

1	CTATCCGCTG	GATGACCAGG	ATGCCATTGC	TGTGGAAGCT	GCCTGCACTA	ATGTTCCGGC
61	GTTATTTCTT	GATGTCTCTG	ACCAGACACC	CATCAACAGT	ATTATTTTCT	CCCATGAAGA
121	CGGTACGCGA	CTGGGCGTGG	AGCATCTGGT	CGCATTGGGT	CACCAGCAAA	TCGCGCTGTT
181	AGCGGGCCCA	TTAAGTTCTG	TCTCGGCGCG	TCTGCGTCTG	GCTGGCTGGC	ATAAATATCT
241	CACTCGCAAT	CAAATTCAGC	CGATAGCGGA	ACGGGAAGGC	GACTGGAGTG	CCATGTCGGG
301	TTTTCAACAA	ACCATGCAAA	TGCTGAATGA	GGGCATCGTT	CCCACTGCGA	TGCTGGTTGC
361	CAACGATCAG	ATGGCGCTGG	GCGCAATGCG	CGCCATTACC	GAGTCCGGGC	TGCGCGTTGG
421	TGCGGATATC	TCGGTAGTGG	GATACGACGA	TACCGAAGAC	AGCTCATGTT	ATATCCCGCC
481	GTC AACACC	ATCAAACAGG	ATTTTCGCCT	GCTGGGGCAA	ACCAGCGTGG	ACCGCTTGCT
541	GCAACTCTCT	CAGGGCCAGG	CGGTGAAGGG	CAATCAGCTG	TTGCCCGTCT	CACGTGGTAA
601	AAGAAAAACC	ACCCTGGCAC	CCAATACGCA	AACCGCCTCT	CCCCGCGCGT	TGGCCGATTTC
661	ATTAATGCAG	CTGGCACGAC	AGGTTTCCCG	ACTGGAAAGC	GGGCAGTGAG	CGCAACGCAA
721	TTAATGTGAG	TTAGCGCGAA	TTGATCTGGT	TTGACAGCTT	ATCATCGACT	GCACGGTGCA
781	CCAATGCTTC	TGGCGTCAGG	CAGCCATCGG	AAGCTGTGGT	ATGGCTGTGC	AGGTCGTAAA
841	TCACTGCATA	ATTCGTGTGG	CTCAAGGCGC	ACTCCCGTTC	TGGATAATGT	TTTTTGCGCC
901	GACATCATAA	CGGTTCTGGC	AAATATTCTG	AAATGAGCTG	TTGACAATTA	ATCATCCGGT
961	CCGTATAATC	TGTGGAATTG	TGAGCGGATA	ACAATTTTAC	ACAGGAAACA	GACCATGGGT
1021	CATCATCATC	ATCATCACGA	TTACGATATC	CCAACGACCG	AAAACCTGTA	TTTTTCAGGGC
1081	GCCCATATGA	GCGATAAAAT	TATTCACCTG	ACTGACGACA	GTTTGGACAC	GGATGTACTC
1141	AAAGCGGACG	GGGCGATCCT	CGTCGATTTC	TGGGCAGAGT	GGTGCGGTCC	GTGCAAAATG
1201	ATCGCCCCGA	TTCTGGATGA	AATCGCTGAC	GAATATCAGG	GCAAACTGAC	CGTTGCAAAA
1261	CTGAACATCG	ATCAAAAACC	TGGCACTGCG	CCGAAATATG	GCATCCGTGG	TATCCCGACT
1321	CTGCTGCTGT	TCAAAAACGG	TGAAGTGGCG	GCAACCAAAG	TGGGTGCACT	GTCTAAAGGT
1381	CAGTTGAAAG	AGTTCCTCGA	CGCTAACCTG	GCCGGTTCTG	GTTCTGGTGA	TGACGATGAC
1441	AAGGTACCCA	TCACAAGTTT	GTACAAAAAA	GCTGAACGAG	AAACGTAAAA	TGATATAAAT
1501	ATCAATATAT	TAAATTAGAT	TTTGCATAAA	AAACAGACTA	CATAATACTG	TAAAACACAA
1561	CATATCCAGT	CACTATGGCG	GCCGCTAAGT	TGGCAGCATC	ACCCGACGCA	CTTTGCGCCG
1621	AATAAATACC	TGTGACGGAA	GATCACTTCG	CAGAATAAAT	AAATCCTGGT	GTCCCTGTGT
1681	ATACCGGGAA	GCCCTGGGCC	AACTTTTGGC	GAAAATGAGA	CGTTGATCGG	CACGTAAGAG
1741	GTTCCAACCT	TCACCATAAT	GAAATAAGAT	CACTACCGGG	CGTATTTTTT	GAGTTATCGA
1801	GATTTTCAGG	AGCTAAGGAA	GCTAAAATGG	AGAAAAAAT	CACTGGATAT	ACCACCGTTG
1861	ATATATCCCA	ATGGCATCGT	AAAGAACATT	TTGAGGCATT	TCAGTCAGTT	GCTCAATGTA
1921	CCTATAACCA	GACCGTTCAG	CTGGATATTA	CGGCCTTTTT	AAAGACCGTA	AAGAAAAATA
1981	AGCACAAAGT	TTATCCGGCC	TTTATTCACA	TTCTTGCCCG	CCTGATGAAT	GCTCATCCGG
2041	AATTCGGTAT	GGCAATGAAA	GACGGTGAGC	TGGTGATATG	GGATAGTGT	CACCTTGTGT
2101	ACATCCGTTT	CCATGAGCAA	ACTGAAACGT	TTTCATCGCT	CTGGAGTGAA	TACCACGACG
2161	ATTTCCGGCA	GTTTCTACAC	ATATATTGCG	AAGATGTGGC	GTGTTACGGT	GAAAACCTGG
2221	CCTATTTCCC	TAAAGGGTTT	ATTGAGAATA	TGTTTTTCGT	CTCAGCCAAT	CCCTGGGTGA
2281	GTTTCACCAG	TTTTGATTTA	AACGTGGCCA	ATATGGACAA	CTTCTTCGCC	CCCGTTTTCA
2341	CCATGGGCAA	ATATTATACG	CAAGGCGACA	AGGTGCTGAT	GCCGCTGGCG	ATTACAGTTC
2401	ATCATGCCGT	CTGTGATGGC	TTCCATGTGC	GCAGAAATGCT	TAATGAATTA	CAACAGTACT
2461	GCGATGAGTG	GCAGGGCGGG	GCGTAAACGC	GTGGATCCGG	CTTACTAAAA	CCAGATAAAC
2521	AGTATGCGTA	TTTGCGCGCT	GATTTTTTGC	GTATAAGAAT	ATATACTGAT	ATGTATACCC-

FIGURE 24B

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2581 GAAGTATGTC AAAAAGAGGT GTGCTATGAA GCAGCGTATT ACAGTGACAG TTGACAGCGA
2641 CAGCTATCAG TTGCTCAAGG CATATATGAT GTCAATATCT CCGGTCTGGT AAGCACAACC
2701 ATGCAGAATG AAGCCCGTCG TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG
2761 ATGGCTGAGG TCGCCCGGTT TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC
2821 TGGTGAAATG CAGTTTAAGG TTTACACCTA TAAAAGAGAG AGCCGTATAT GTCTGTTTGT
2881 GGATGTACAG AGTGATATTA TTGACACGCC CGGGCGACGG ATGGTGATCC CCCTGGCCAG
2941 TGCACGTCTG CTGTCAGATA AAGTCTCCCG TGAACCTTAC CCGGTGGTGC ATATCGGGGA
3001 TGAAAGCTGG CGCATGATGA CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA
3061 AGAAGTGGCT GATCTCAGCC ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT
3121 CTGGCGAATA TAAATGTCAG GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG
3181 ACTGGATATG TTGTGTTTTA CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA
3241 ATATATTGAT ATTTATATCA TTTTACGTTT CTCGTTTCAGC TTTCTTGATC AAAGTGGTGA
3301 TGGGGATCCT CTAGAGTCGA CCTGCAGTAA TCGTACAGGG TAGTACAAAT AAAAAAGGCA
3361 CGTCAGATGA CGTGCCCTTT TTCTTGAGAG CAGTAAGCTT GGCTGTTTTG GCGGATGAGA
3421 GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA TAAAACAGAA
3481 TTTGCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAAC CAGAAGTGAA
3541 ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCATGCG AGAGTAGGGA ACTGCCAGGC
3601 ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT TCGTTTTATC TGTGTTTGT
3661 CGGTGAACGC TCTCCTGAGT AGGACAAATC CGCCGGGAGC GGATTTGAAC GTTGCGAAGC
3721 AACGCCCCGG AGGGTGGCGG GCAGGACGCC CGCCATAAAC TGCCAGGCAT CAAATTAAGC
3781 AGAAGGCCAT CCTGACGGAT GGCTTTTTTG CGTTTCTACA AACTCTTTTT GTTTATTTTT
3841 CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
3901 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT
3961 TGCGGCATTT TGCCTTCCTG TTTTGTCTCA CCCAGAAACG CTGGTGAAAG TAAAAGATGC
4021 TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT
4081 CCTTGAGAGT TTTGCCCCCG AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT
4141 ATGTGGCGCG GTATTATCCC GTGTTGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
4201 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG
4261 CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG AGTGATAACA CTGCGGCCAA
4321 CTATTCTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG
4381 GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAACGGA
4441 CGAGCGTGAC ACCACGATGC CTACAGCAAT GGCAACAACG TTGCGCAAAC TATTAAGTGG
4501 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT
4561 TGCAAGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG TTTATTGCTG ATAAATCTGG
4621 AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC
4681 CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA
4741 GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
4801 ATATATACTT TAGATTGATT TAAAACCTCA TTTTAAATTT AAAAGGATCT AGGTGAAGAT
4861 CCTTTTTGAT AATCTCATGA CCAAATCCC TTAACGTGAG TTTTCGTTCC ACTGAGCGTC
4921 AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTCTGCG GCGTAATCTG
4981 CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTGTCGGG ATCAAGAGCT
5041 ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
5101 TCTAGTGTAG CCGTAGTTAG GCCACCCTT CAAGAACTCT GTAGCACCAG CTACATACCT
5161 CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGCG GATAAGTCGT GTCTTACCGG
5221 GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTT
5281 GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA
5341 GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
5401 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA
5461 TAGTCTGTG GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTGTGAT GCTCGTCAGG
5521 GGGGCGGAGC CTATGAAAAA ACGCCAGCAA CGCGGCTTTT TTACGGTTCC TGGCCTTTTG
5581 CTGGCCTTTT GCTCACATGT TCTTCTGTC GTTATCCCTT GATTCTGTGG ATAAACGTAT
5641 TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
5701 AGTGAGCGAG GAAGCGGAAG AGCGCCTGAT GCGGTATTTT CTCCTTACGC ATCTGTGCGG
5761 TATTTACACAC CGCATAATTT TGTTAAATTT CGCGTTAAAT TTTTGTGAAA TCAGCTCATT
5821 TTTTAACCAA TAGGCCGAAA TCGGCAAAAT CCCTTATAAA TCAAAAGAAAT AGACCGAGAT
5881 AGGGTTGAGT GTTGTTCAG TTTGGAACAA GAGTCCACTA TTAAAGAACG TGGACTCCAA
5941 CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA CTACGTGAAC CATCACCTTA
6001 ATCAAGTTTT TTGGGTTCGA GGTGCCGTAA AGCACTAAAT CGGAACCCTA AAGGGAGCCC-

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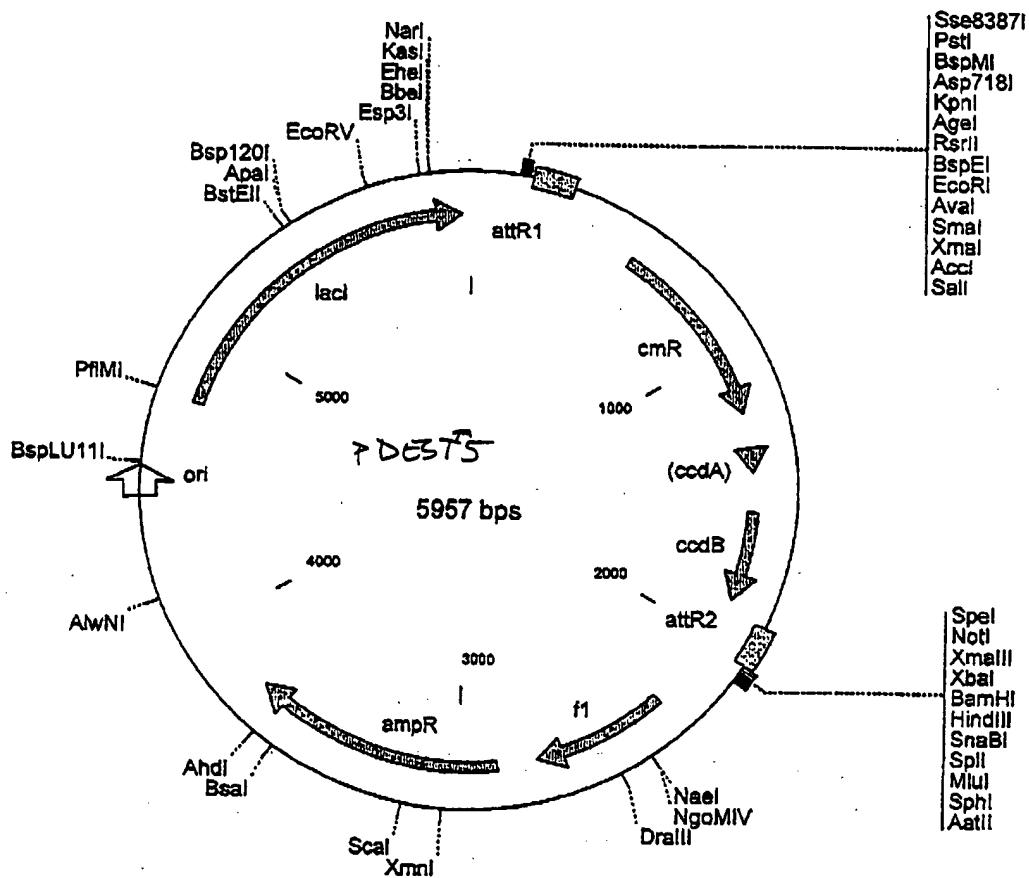
FIGURE 24C

6061 CCGATTTAGA GCTTGACGGG GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC  
6121 GAAAGGAGCG GGCGCTAGGG CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG TAACCACCAC  
6181 ACCCGCCGCG CTTAATGCGC CGCTACAGGG CGCGTCCATT CGCCATTTCAG GCTGCTATGG  
6241 TGCACTCTCA GTACAATCTG CTCTGATGCC GCATAGTTAA GCCAGTATAC ACTCCGCTAT  
6301 CGCTACGTGA CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT  
6361 GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC TCCGGGAGCT  
6421 GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGCAG ATCAATTTCG  
6481 GCGCGAAGGC GAAGCGGCAT GCATTACGT TGACACCATC GAATGGTGCA AAACCTTTG  
6541 CGGTATGGCA TGATAGCGCC CGGAAGAGAG TCAATTTCAG GTGGTGAATG TGAAACCACT  
6601 AACGTTATAC GATGTCGCAG AGTATGCCGG TGTCTCTTAT CAGACCGTTT CCGCGTGGT  
6661 GAACCAGGCC AGCCACGTTT CTGCGAAAAC GCGGGAAAAA GTGGAAGCGG CGATGGCGGA  
6721 GCTGAATTAC ATTCCCAACC GCGTGGCACA ACAACTGGCG GGCAAACAGT CGTTGCTGAT  
6781 TGGCGTTGCC ACCTCCAGTC TGGCCCTGCA CGCGCCGTCG CAAATTGTCG CGGCGATTAA  
6841 ATCTCGCGCC GATCAACTGG GTGCCAGCGT GGTGGTGTG ATGGTAGAAC GAAGCGCGCT  
6901 CGAAGCCTGT AAAGCGGCGG TGCACAATCT TCTCGCGCAA CGCGTCAGTN GGGCTGATCA  
6961 TTAA



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Figure 25B  $\gamma$ DEST5 (cont'd)



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## pDEST5 5957 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
305..181		attR1
555..1214		CmR
1334..1418		inactivated ccdA
1556..1861		ccdB
1902..2026		attR2
2278..2733		f1 (f1 intergenic region)
2865..3722		ampR
5378..5538		ori
4756..5922		lacI

1	AGGCACCCCA	GGCTTTACAC	TTTATGCTTC	CGGCTCGTAT	GTTGTGTGGA	ATTGTGAGCG
61	GATAACAATT	TCACACAGGA	AACAGCTATG	ACCATGATTA	CGCCAAGCTC	TAATACGACT
121	CACTATAGGG	AAAGCTGGTA	CGCCTGCAGG	TACCGGTCCG	GAATTCCTCG	GTCGACGATC
181	ACAAGTTTGT	ACAAAAAAGC	TGAACGAGAA	ACGTAAATG	ATATAAATAT	CAATATATTA
241	AATTAGATTT	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA
301	CTATGGCGGC	CGCTAAGTTG	GCAGCATCAC	CCGACGCACT	TGCGCCGAA	TAAATACCTG
361	TGACGGAAGA	TCACTTCGCA	GAATAAATAA	ATCCTGGTGT	CCCTGTTGAT	ACCGGGAAGC
421	CCTGGGCCAA	CTTTTGGCGA	AAATGAGACG	TTGATCGGCA	CGTAAGAGGT	TCCAACCTTC
481	ACCATAATGA	AATAAGATCA	CTACCGGGCG	TATTTTGTGA	GTTATCGAGA	TTTTTCAGGAG
541	CTAAGGAAGC	TAAAATGGAG	AAAAAATCA	CTGGATATAC	CACCGTTGAT	ATATCCCAAT
601	GGCATCGTAA	AGAACATTTT	GAGGCATTTT	AGTCAGTTGC	TCAATGTACC	TATAACCAGA
661	CCGTTTCAGCT	GGATATTACG	GCCTTTTAA	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT
721	ATCCGGCCTT	TATTCACATT	CTTGCCCGCC	TGATGAATGC	TCATCCGGAA	TTCCGTATGG
781	CAATGAAAGA	CGGTGAGCTG	GTGATATGGG	ATAGTGTTCA	CCCTTGTTAC	ACCGTTTTCC
841	ATGAGCAAAC	TGAAACGTTT	TCATCGTCTT	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT
901	TTCTACACAT	ATATTGCAAA	GATGTGGCGT	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA
961	AAGGGTTTTAT	TGAGAATATG	TTTTTCGTCT	CAGCCAATCC	CTGGGTGAGT	TTCAACAGTT
1021	TTGATTTAAA	CGTGGCCAAT	ATGGACAAC	TCTTCGCCCC	CGTTTTTACC	ATGGGCAAA
1081	ATTATACGCA	AGGCGACAAG	GTGCTGATGC	CGCTGGCGAT	TCAGGTTTAT	CATGCCGTCT
1141	GTGATGGCTT	CCATGTGCGC	AGAATGCTTA	ATGAATTACA	ACAGTACTGC	GATGAGTGGC
1201	AGGGCGGGGC	GTAACGCGT	GGATCCGGCT	TACTAAAAGC	CAGATAACAG	TATGCGTATT
1261	TGCGCGCTGA	TTTTTGCGGT	ATAAGAATAT	ATACTGATAT	GTATACCGGA	AGTATGTCAA
1321	AAAGAGGTGT	GCTATGAAGC	AGCGTATTAC	AGTGACAGTT	GACAGCGACA	GCTATCAGTT
1381	GCTCAAGGCA	TATATGATGT	CAATATCTCC	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA
1441	GCCCGTTCGTC	TGCGTGCCGA	ACGCTGGAAA	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC
1501	GCCCGGTTTA	TTGAAATGAA	CGGCTCTTTT	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA
1561	GTTTAAGGTT	TACACCTATA	AAAGAGAGAG	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG
1621	TGATATTATT	GACACGCCCG	GGCGACGGAT	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT
1681	GTCAGATAAA	GTCTCCCGTG	AACTTTACCC	GGTGGTGTCAT	ATCGGGGATG	AAAGCTGGCG
1741	CATGATGACC	ACCGATATGG	CCAGTGTGCC	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA
1801	TCTCAGCCAC	CGCGAAAATG	ACATCAAAAA	CGCCATTAAC	CTGATGTTCT	GGGGAATATA
1861	AATGTCAGGC	TCCCTTATAC	ACAGCCAGTC	TGCAGGTCGA	CCATAGTGAC	TGGATATGTT
1921	GTGTTTTTACA	GTATTATGTA	GTCTGTTTTT	TATGCAAAAT	CTAATTTAAT	ATATTGATAT
1981	TTATATCATT	TTACGTTTCT	CGTTCAGCTT	TCTTGTAACA	AGTGGTGATC	ACTAGTCGGC
2041	GGCCGCTCTA	GAGGATCCAA	GCTTACGTAC	GCGTGCAATG	GACGTCATAG	CTCTTCTATA
2101	GTGTCACCTA	AATTCAATTC	ACTGGCCGTC	GTTTTACAAC	GTCGTGACTG	GGAAAACCC
2161	GGCGTTACCC	AACTTAATCG	CCTTGACGCA	CATCCCCCTT	TCGCCAGCTG	GCGTAATAGC
2221	GAAGAGGCCC	GCACCGATCG	CCCTTCCCAA	CAGTTGCGCA	GCCTGAATGG	CGAATGGACG
2281	CGCCCTGTAG	CGGCGCATT	AGCGCGGCGG	GTGTGGTGGT	TACGCGCAGC	GTGACCGCTA
2341	CACCTTGCCAG	CGCCCTAGCG	CCGCTTCTTT	TCGCTTTCTT	CCCTTCTTTT	CTCGCCACGT
2401	TCCGCGGCTT	TCCCGTCAA	GCTCTAAATC	GGGGGCTCCC	TTTAGGGTTC	CGATTAGTG
2461	CTTTACGGCA	CCTCGACCCC	AAAAAACTTG	ATTAGGGTGA	TGGTTCACGT	AGTGGGCCAT
2521	CGCCCTGATA	GACGGTTTTT	CGCCCTTTGA	CGTTGGAGTC	CACGTTCTTT	AATAGTGGAC
2581	TCTTGTTCCA	AACTGGAACA	AACTCAACC	CTATCTCGGT	CTATTCTTTT	GATTTATAAG-

FIGURE 25C

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2641 GGATTTTGGCC GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG  
2701 CGAATTTTAA CAAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG GGAAATGTGC  
2761 GCGGAACCCC TATTTGTTTA TTTTCTAAA TACATTCAA TATGTATCCG CTCATGAGAC  
2821 AATAACCCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCAACATT  
2881 TCCGTGTGCG CCTTATTCCC TTTTTCGGG CATTTCGCCT TCCTGTTTTT GCTCACCCAG  
2941 AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTTCATCG  
3001 AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCGAAGAA CGTTTTCCAA  
3061 TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT GACGCCGGGC  
3121 AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA CTGCGTTGAG TACTCACCAG  
3181 TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA  
3241 CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC  
3301 TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACCTG CTTGATCGT TGGGAACCGG  
3361 AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA  
3421 CAACGTTGCG CAAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA  
3481 TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGCTG  
3541 GCTGGTTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTCAG  
3601 CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG  
3661 CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT  
3721 GGTAACGTG AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCAATTTT  
3781 AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAA ATCCCTTAAC  
3841 GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG  
3901 ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAAACAA AAAACCACCG CTACCAGCGG  
3961 TGGTTTTGTTT GCCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAAT GGCTTCAGCA  
4021 GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGAGCCGTA GTTAGGCCAC CACTTCAAGA  
4081 ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA  
4141 GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC  
4201 AGCGGTGCGG CTGAACGGGG GGTTCGTGCA CACAGCCAG CTTGGAGCGA ACGACCTACA  
4261 CCGAAGTGA ATACCTACAG CGTGAGCATT GAGAAAGCG CACGCTTCCC GAAGGGAGAA  
4321 AGGCGGACAG GTATCCGTA AGCGGCAGGG TCGGAACAGG AGAGCGCAG AGGGAGCTTC  
4381 CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTGCGGTT TCGCCACCTC TGACTTGAGC  
4441 GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACCGCG  
4501 CCTTTTTTACG GTTCTTGGCC TTTTGCTGGC CTTTGTCTCA CATGTTCTTT CTGCGTTAT  
4561 CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC GCTCGCCGCA  
4621 GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC CCAATACGCA  
4681 AACCGCCTCT CCCCAGCGCT TGGCCGATTC ATTAATGCAG AGCTTGCAAT TCGCGCGCGA  
4741 AGGCGAAGCG GCATTTACGT TGACACCATC GAATGGCGCA AAACCTTTTC CGGTATGGCA  
4801 TGATAGCGCC CGGAAGAGAG TCAATTCAGG GTGGTGAATG TGAAACAGT AACGTTATAC  
4861 GATGTGCGAG AGTATGCCGG TGTCTCTTAT CAGACCGTTT CCCGCGTGGT GAACAGGCC  
4921 AGCCACGTTT CTGCGAAAAC GCGGGAAAAA GTGGAAGCGG CGATGGCGGA GCTGAATTAC  
4981 ATTCCCAACC CCGTGGCACA ACAACTGGCG GGCAAACAGT CGTTGCTGAT TGGCGTTGCC  
5041 ACCTCCAGTC TGGCCCTGCA CGCGCCGTCG CAAATTGTCT GCGCGATTAA ATCTCGCGCC  
5101 GATCAACTGG GTGCCAGCGT GGTGGTGTCT ATGGTAGAAC GAAGCGGCGT CGAAGCCTGT  
5161 AAAGCGGCGG TGCACAATCT TCTCGCGCAA CGGGTCAGTG GGCTGATCAT TAACATCCG  
5221 CTGGATGACC AGGATGCCAT TGCTGTGGAA GCTGCCTGCA CTAATGTTCC GGCGTTATTT  
5281 CTTGATGTCT CTGACCAGAC ACCCATCAAC AGTATTATTT TCTCCCATGA AGACGGTACG  
5341 CGACTGGGCG TGGAGCATCT GGTGCGATTG GGTCAACAGC AAATCGCGCT GTTAGCGGGC  
5401 CCATTAAGTT CTGTCTCGGC GCGTCTGCGT CTGGCTGGCT GGCATAAATA TCTCACTCGC  
5461 AATCAAATTC AGCCGATAGC GGAACGGGAA GCGCACTGGA GTGCCATGTC CGGTTTTCAA  
5521 CAAACCATGC AAATGCTGAA TGAGGGCATC GTTCCCACTG CGATGCTGGT TGCCAACGAT  
5581 CAGATGGCGC TGGGCGCAAT GCGCGCCATT ACCGAGTCCG GGCTGCGCGT TGGTGGCGAT  
5641 ATCTCGGTAG TGGGATACGA CGATACCGAA GACAGCTCAT GTTATATCCC GCCGTCAACC  
5701 ACCATCAAAC AGGATTTTCG CCTGCTGGGG CAAACAGCG TGGACCGCTT GCTGCAACTC  
5761 TCTCAGGGCC AGGCGGTGAA GGGCAATCAG CTGTTGCCCC TCTCACTGGT GAAAAAGAAA  
5821 ACCACCTGG CGCCCAATAC GCAAACCGCC TCTCCCCGCG CGTTGGCCGA TTCATTAATG  
5881 CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT  
5941 GAGTTAGCTC ACTCAT

FIGURE 25D

Figure 26A

pDET6

pSPORT "4"

(opposite strand)

## "forward" sequencing primers

1 taa tgc cag ggt ttt ccc agt cac gac gtt gta aaa cga cgg cca gtg aat  
att gcg gtc cca aaa ggg tca gtg ctg caa cat ttt gct gcc ggt cac tta

52 tga att tag gtg aca cta tag aag agc tat gac gtc gca tgc acg cgt acg  
act tta atc cac tgt gat atc ttc tgc ata ctg cag ggt acg tgc gca tgc

SP6 promoter Sph Mlu

103 tca gct tgg atc ctc tag agc ggc cgc cga cta gtg atc aca agt ctg tga  
att cga acc tag gag atc tgc cgc ggc gct gat cac tag tgt tca aac atg

Hind3 Bam Xba Not Spe Xba1 Int

154 aaa aaa gct gaa cga gaa acg taa aat gar ata aat atc aat ata taa aat  
ttt tat cga ctt gct ctt tgc att tta cta tat tca tag cta tat aat tca

↓ Gene

1939 tat tta tat tat ttt acg att cct gtt tag ctt tct tgt aca aag tgg tga  
ata dat ata gta aaa tgc aaa gag gaa gtc gaa aga gca tgc ttc acc att

Int att R2

1990 tgc tgc acc cgg gaa ttc cgg acc ggt act tgc agg cgt acc agc ttt ccc  
agc agc ggg gcc ctt aag gcc tgg dca tgg acg tcc gca tgg tgc aaa ggg

Sal Sph EcoRI Kpn Pst

T7 RNA

2041 tat agt gag tgc tat tag agc ttg gcg taa tca tgg tca tag ctg ttt cct  
ata tca ctc agc ata atc tgc aac cgc att agt acc agt atc gac aaa gga

T7 promoter α-peptide

2092 gtg tga aat tgt tat ccg ctc aca att cca cac aac ata cga gcc gga agc  
cac act tta aca ata ggc gag tgt taa ggt gtg ttg tat gct cgg cct tgc

... sequencing primers lac promoter

lac RNA

2143 ata aag tgt aaa gcc tgg ggt gcc taa tga gtg agc taa ctc aca tta att  
tat ttc aca ttc cgg acc cca cgg att act cac tgc att gag tgt aat taa

-35

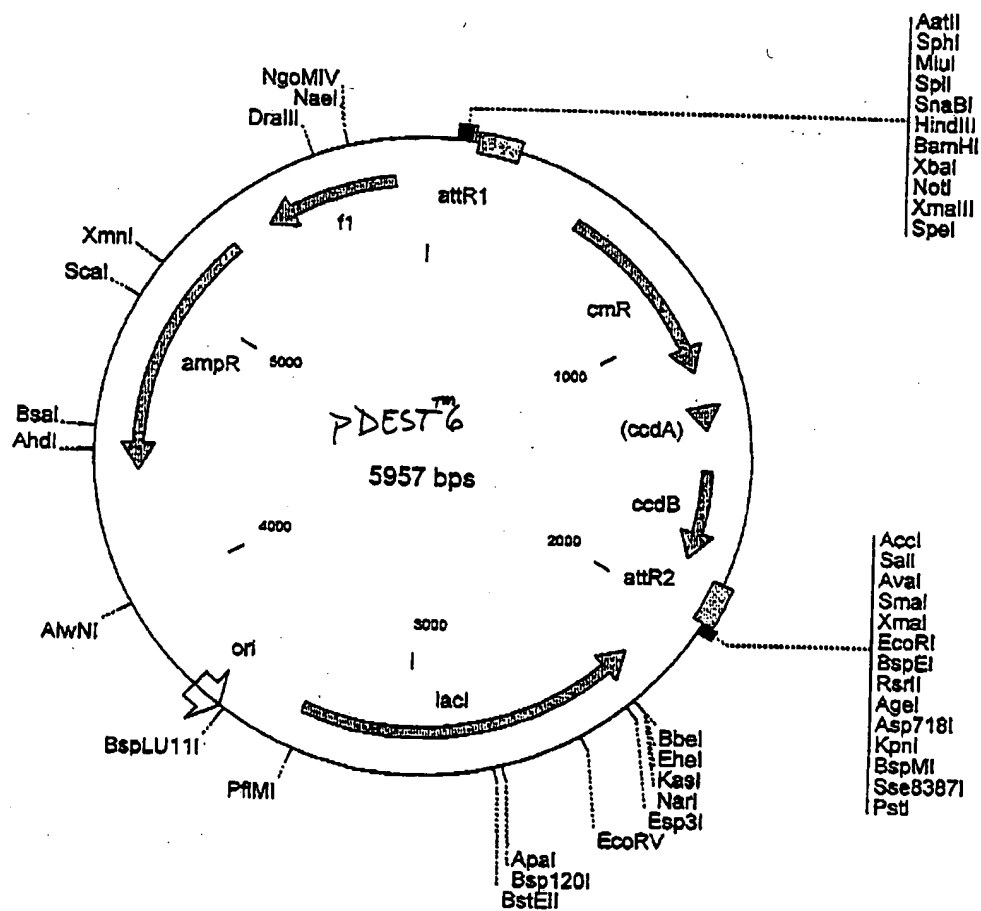


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Figure 26B

PDEST6

(cont'd)



## pDEST6 5957 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
266..142		attR1
516..1175		CmR
1295..1379		inactivated ccdA
1517..1822		ccdB
1863..1987		attR2
2203..3369		lacI
4403..5260		ampR
5392..5847		f1 (f1 intergenic region)

1	TAACGCCAGG	GTTTTCCCAG	TCACGACGTT	GTAAAACGAC	GGCCAGTGAA	TTGAATTTAG
61	GTGACACTAT	AGAAGAGCTA	TGACGTCGCA	TGCACGCGTA	CGTAAGCTTG	GATCCTCTAG
121	AGCGGCCGCC	GACTAGTGAT	CACAAGTTTG	TACAAAAAAG	CTGAACGAGA	AACGTAAAAAT
181	GATATAAATA	TCAATATATT	AAATTAGATT	TTGCATAAAA	AACAGACTAC	ATAATACTGT
241	AAAACACAAC	ATATCCAGTC	ACTATGGCGG	CCGCTAAGTT	GGCAGCATCA	CCCGACGCAC
301	TTTGCGCCGA	ATAAATACCT	GTGACGGAAG	ATCACTTCGC	AGAATAAATA	AATCCTGGTG
361	TCCCTGTTGA	TACCGGGAAG	CCCTGGGCCA	ACTTTTGGCG	AAAATGAGAC	GTTGATCGGC
421	ACGTAAGAGG	TTCCAACTTT	CACCATAATG	AAATAAGATC	ACTACCGGGC	GTATTTTTTG
481	AGTTATCGAG	ATTTTCAGGA	GCTAAGGAAG	CTAAATGGA	GAAAAAATC	ACTGGATATA
541	CCACCGTTGA	TATATCCCAA	TGGCATCGTA	AAGAACATT	TGAGGCATT	CAGTCAGTTG
601	CTCAATGTAC	CTATAACCAG	ACCGTTCAGC	TGGATATTAC	GGCCTTTT	AAGACCGTAA
661	AGAAAAATAA	GCACAAGTTT	TATCCGGCCT	TTATTCACAT	TCTTGCCCGC	CTGATGAATG
721	CTCATCCGGA	ATTCCGTATG	GCAATGAAAG	ACGGTGAGCT	GGTGATATGG	GATAGTGTTT
781	ACCCTTGTTA	CACCGTTTTT	CATGAGCAAA	CTGAAACGTT	TTCATCGCTC	TGGAGTGAAT
841	ACCACGACGA	TTTCCGGCAG	TTTCTACACA	TATATTCGCA	AGATGTGGCG	TGTTACGGTG
901	AAAACCTGGC	CTATTTCCCT	AAAGGGTTTA	TTGAGAATAT	GTTTTTCGTC	TCAGCCAATC
961	CCTGGGTGAG	TTTCACCAGT	TTTGATTTAA	ACGTGGCCAA	TATGGACAAC	TTCTTCGCCC
1021	CCGTTTTTAC	CATGGGCAAA	TATTATACGC	AAGGCGACAA	GGTGCTGATG	CCGCTGGCGA
1081	TTCAGGTTCA	TCATGCCGTC	TGTGATGGCT	TCCATGTCGG	CAGAATGCTT	AATGAATTAC
1141	AACAGTACTG	CGATGAGTGG	CAGGGCGGGG	CGTAAACGCG	TGGATCCGGC	TTACTAAAAG
1201	CCAGATAACA	GTATGCCGAT	TTGCGCGCTG	ATTTTTGCGG	TATAAGAATA	TATACTGATA
1261	TGTATACCCG	AAGTATGTCA	AAAAGAGGTG	TGCTATGAAG	CAGCGTATTA	CAGTGACAGT
1321	TGACAGCGAC	AGCTATCAGT	TGCTCAAGGC	ATATATGATG	TCAATATCTC	CGGTCTGGTA
1381	AGCACAACCA	TGCAGAAATG	AGCCCGTCGT	CTGCGTGCCG	AACGCTGGAA	AGCGGAAAAAT
1441	CAGGAAGGGA	TGGCTGAGGT	CGCCCGGTTT	ATTGAAATGA	ACGGCTCTTT	TGCTGACGAG
1501	AACAGGGACT	GGTGAAATGC	AGTTTAAAGT	TTACACCTAT	AAAAGAGAGA	GCCGTTATCG
1561	TCTGTTTGTG	GATGTACAGA	GTGATATTAT	TGACACGCCC	GGGCGACGGA	TGGTGATCCC
1621	CCTGGCCAGT	GCACGTCTGC	TGTCAGATAA	AGTCTCCCGT	GAACCTTACC	CGGTGGTGCA
1681	TATCGGGGAT	GAAAGCTGGC	GCAATGATGAC	CACCGATATG	GCCAGTGTGC	CGGTCTCCGT
1741	TATCGGGGAA	GAAGTGGCTG	ATCTCAGCCA	CCGCGAAAAAT	GACATCAAAA	ACGCCATTAA
1801	CCTGATGTTT	TGGGGAATAT	AAATGTCAGG	CTCCCTTATA	CACAGCCAGT	CTGCAGGTCT
1861	ACCATAGTGA	CTGGATATGT	TGTGTTTTAC	AGTATTATGT	AGTCTGTTTT	TTATGCAAAA
1921	TCTAATTTAA	TATATTGATA	TTTATATCAT	TTTACGTTTC	TCGTTACAGT	TTCTTGATCA
1981	AAGTGGTGAT	CGTCGACCCG	GGAATTCGCG	ACCGGTACCT	GCAGGCGTAC	CAGCTTTCCC
2041	TATAGTGAGT	CGTATTAGAG	CTTGGCGTAA	TCATGGTCAT	AGCTGTTTTCC	TGTGTGAAAT
2101	TGTTATCCGC	TCACAATTCC	ACACAACATA	CGAGCCGGAA	GCATAAAGTG	TAAAGCCTGG
2161	GGTGCCCTAAT	GAGTGAGCTA	ACTCACATTA	ATTGCGTTGC	GCTCACTGCC	CGCTTTCCAG
2221	TCGGGAAACC	TGTCGTGCCA	GCTGCATTAA	TGAATCGGCC	AACGCGCGGG	GAGAGGCGGT
2281	TTGCGTATTG	GGCGCCAGGG	TGGTTTTTCT	TTTCACCACT	GAGACGGGCA	ACAGCTGATT
2341	GCCCTTCACC	GCCTGGCCCT	GAGAGAGTTG	CAGCAAGCGG	TCCACGCTGG	TTTGCCCCAG
2401	CAGGCGAAAA	TCCTGTTTGA	TGGTGGTTGA	CGGCGGGATA	TAACATGAGC	TGCTTCCGGT
2461	ATCGTCGTAT	CCCACTACCG	AGATATCCGC	ACCAACGCGC	AGCCCGGACT	CGGTAATGGC
2521	GCGCATTGCG	CCCAGCGCCA	TCTGATCGTT	GGCAACCAGC	ATCGCAGTGG	GAACGATGCC
2581	CTCATTCAGC	ATTTGCATGG	TTTGTGAAAT	ACCGGACATG	GCACTCCAGT	CGCCTTCCCG
2641	TTCCGCTATC	GGCTGAATTT	GATTGCGAGT	GAGATATTTA	TGCCAGCCAG	CCAGACCCAG

FIGURE 26C

2701 ACGCGCCGAG ACAGAACTTA ATGGGCCCCG TAACAGCGCG ATTTGCTGGT GACCCAATGC  
2761 GACCAGATGC TCCACGCCCA GTCGCGTACC GTCTTCATGG GAGAAAATAA TACTGTTGAT  
2821 GGGTGTCTGG TCAGAGACAT CAAGAAATAA CGCCGGAACA TTAGTGACAGG CAGCTTCCAC  
2881 AGCAATGGCA TCCTGGTCAT CCAGCGGATA GTTAATGATC AGCCCACTGA CCCGTTGCGC  
2941 GAGAAGATTG TGCACCGCCG CTTTACAGGC TTGACGCGG CTTCGTTCTA CCATCGACAC  
3001 CACCACGCTG GCACCCAGTT GATCGGCGCG AGATTTAATC GCCGCGACAA TTTGCGACGG  
3061 CGCGTGCAGG GCCAGACTGG AGGTGGCAAC GCCAATCAGC AACGACTGTT TGCCCCCAG  
3121 TTGTTGTGCC ACGCGGTGG GAATGTAATT CAGCTCCGCC ATCGCCGCTT CCACTTTTC  
3181 CCGCGTTTTT GCAGAAACGT GGCTGGCCTG GTTCACCACG CGGGAACGG TCTGATAAGA  
3241 GACACCGGCA TACTCTGCGA CATCGTATAA CGTTACTGGT TTCACATTCA CCACCCTGAA  
3301 TTGACTCTCT TCCGGGCGCT ATCATGCCAT ACCGCGAAAG GTTTTGCGCC ATTCGATGGT  
3361 GTCAACGTAA ATGCCGCTTC GCCTTCGCGC GCGAATTGCA AGCTCTGCAT TAATGAATCG  
3421 GCCAACGCGC GGGGAGAGGC GGTTCGCGTA TTGGGCGCTC TTCCGCTTCC TCGCTCACTG  
3481 ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA AAGCGGTAG  
3541 TACGGTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC  
3601 AAAAGGCCAG GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC  
3661 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT  
3721 AAAGATACCA GGCCTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT CCGACCCTGC  
3781 CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCCGGGAG CGTGGCGCTT TCTCAATGCT  
3841 CACGCTGTAG GTATCTCAGT TCGGTGTAGG TCGTTTCGCTC CAAGCTGGGC TGTGTGCACG  
3901 AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC  
3961 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA  
4021 GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC TACACTAGAA  
4081 GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCCGAAAA AGAGTTGGTA  
4141 GCTCTTGATC CGGCAACAAA ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC  
4201 AGATTACGCG CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG  
4261 ACGTCTCAGT GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA  
4321 TCTTCACTTA GATCCTTTTA AATTAAAAAT GAAGTTTAA ATCAATCTAA AGTATATATG  
4381 AGTAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC TCAGCGATCT  
4441 GTCTATTTTC TTTATCCATA GTTGCTGAC TCCCGTCGT GTAGATAACT ACGATACGGG  
4501 AGGCTTACC ATCTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC  
4561 CAGATTTATC AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCTGCAA  
4621 CTTATCCCG CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTGCG  
4681 CAGTTAATAG TTTGCGCAAC GTTGTGGCCA TTGCTACAGG CATCGTGGTG TCACGCTCGT  
4741 CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC AAGGCGAGTT ACATGATCCC  
4801 CCATGTTGTG CAAAAAGCG GTTAGCTCCT TCGGTCTCTC GATCGTTGTC AGAAGTAAGT  
4861 TGCCCGCAGT GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTATGTC  
4921 CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT  
4981 GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC GCGCCACATA  
5041 GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA CTCTCAAGGA  
5101 TCTTACCGCT GTTGAGATCC AGTTCGATGT AACCCTACTG TGCACCCAAC TGATCTTCAG  
5161 CATCTTTTAC TTTACCAGC GTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA  
5221 AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT  
5281 ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA TGTATTTAGA  
5341 AAAATAACA AATAGGGGTT CCGCGCACAT TTCCCGAAA AGTGCCACCT GAAATTTGTA  
5401 ACGTTAATAT TTTGTTAAAA TTCGCGTTAA ATTTTGTGTA AATCAGCTCA TTTTAAACC  
5461 AATAGGCCGA AATCGGCAAA ATCCCTTATA AATCAAAAGA ATAGACCGAG ATAGGGTTGA  
5521 GTGTTGTTCC AGTTTGAAC AAGAGTCCAC TATTAAGAA CGTGGACTCC AACGTCAAAG  
5581 GGCGAAAAAC CGTCTATCAG GCGGATGGCC CACTACGTGA ACCATCACCC TAATCAAGTT  
5641 TTTTGGGGTC GAGGTGCCGT AAAGCACTAA ATCGGAACCC TAAAGGGAGC CCCCATTATA  
5701 GAGCTTGACG GGGAAAGCCG GCGAACGTGG CGAGAAAGGA AGGGAAGAAA GCGAAAGGAG  
5761 CGGGCGCTAG GCGCTGGCA AGTGTAGCGG TCACGTGCGG CGTAACCACC ACACCCCGCG  
5821 CGCTTAATGC CCGCTACAG GCGCGTGCCA TTCGCCATTC AGGCTGCGCA ACTGTTGGGA  
5881 AGGGCGATCG GTGCGGGCCT CTTGCTATT ACGCCAGCTG GCGAAAGGGG GATGTGCTGC  
5941 AAGGCGATTA AGTTGGG

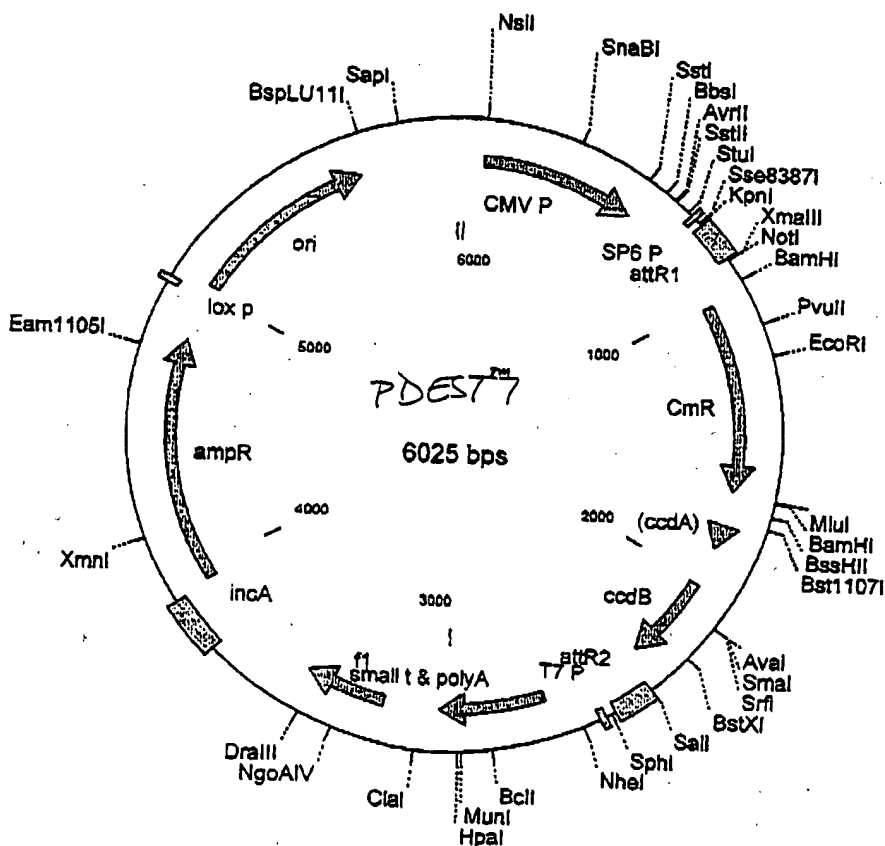
FIGURE 26D

Figure 27A: PDEST7

## CMV promoter for eukaryotic expression

970 cca ttg acg caa atg ggc ggt agg cgt gta cgg tgg gag gtc tat ata agc  
 ggt aac tgc gtt tac ccg cca tcc gca cat gcc acc ctc cag ata tat tcg  
 1021 aga gct cgt tta gtg aac cgt cag atc gcc tgg aga cgc cat cca cgc tgt  
 tct cga gca aat cac ttg gca gtc tag cgg acc tct gcg gta ggt gcg aca  
 1072 ttt gac ctc cat aga aga cac cgg gac cga tcc agc ctc cgg act cta gcc  
 aaa ctg gag gta tct tct gtg gcc ctg gct agg tgg gag ggc tga gat cgg  
 1123 tag gcc gcg gag cgg ata aca att tca cac agg aaa cag cta tga cca cta  
 atc cgg cgc ctc gcc tat tgt taa agt gtg tcc ttt gtc gat act ggt gat  
 1174 ggc ttt tgc aaa aag cta ttt agg tga cac tat aga agg tac gcc tgc agg  
 ccg aaa acg ttt ttc gat aaa tcc act gtg ata tct tcc atg cgg acg tct  
 1225 tac cgg tcc gga att ccc atc aca agt tgg tag aaa gaa ggt gaa cga gaa  
 atg gcc agg cct taa ggg tag tgt tca aac atg ttt tct cga ctc gct ctc

Annotations:   
 - mRNA start (arrow) at position 970  
 - CMV enhancer / promoter (arrow) at position 1021  
 - EcoRI (arrow) at position 1072  
 - KpnI (arrow) at position 1123  
 - PstI (arrow) at position 1174  
 - NotI (arrow) at position 1225



## pDEST7 6025 bp (rotated to position 2800)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
67..589	CMV promoter
906..782	attR1
1015..1674	CmR
1794..1878	inactivated ccdA
2016..2321	ccdB
2362..2486	attR2
2671..3033	small t & polyA
3227..3502	fl
3962..4822	ampR
5022..5661	ori

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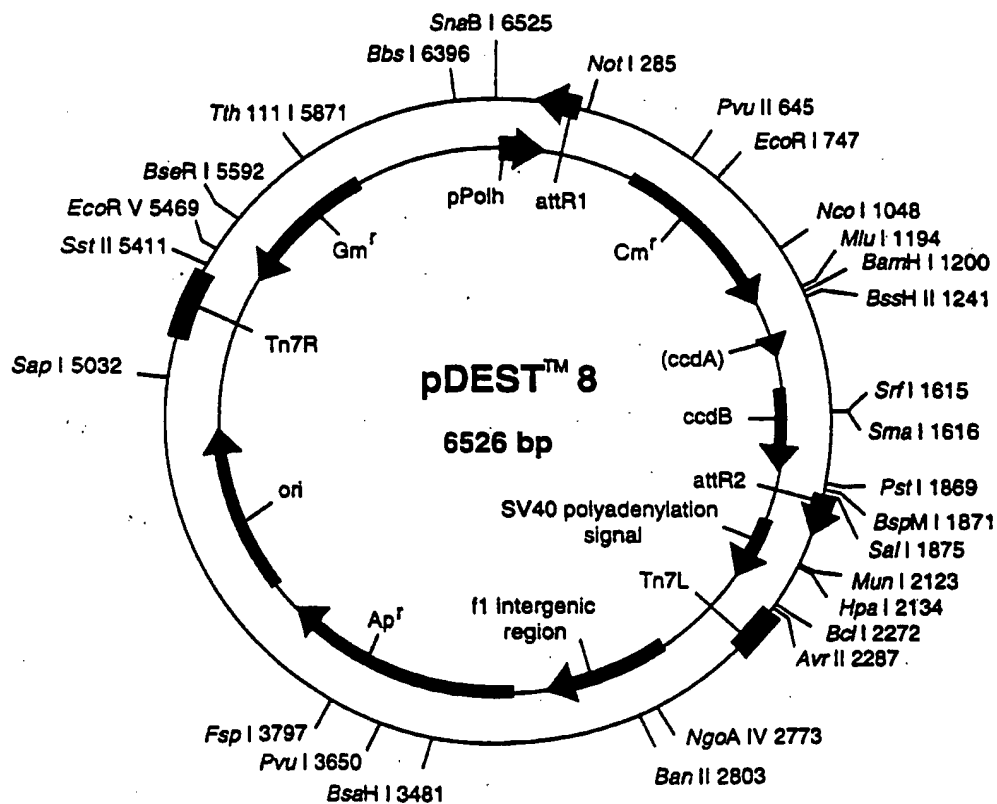
1  ATTATCATGA  CATTAACTTA  TAAAAATAGG  CGTAGTACGA  GGCCCTTTCA  CTCATTAGAT
61 GCATGTCGTT  ACATAACTTA  CGGTAAATGG  CCCGCCTGGC  TGACCGCCCA  ACGACCCCGG
121 CCCATTGACG  TCAATAATGA  CGTATGTTCC  CATAGTAAAG  CCAATAGGGA  CTTTCCATTG
181 ACGTCAATGG  GTGGAGTATT  TACGGTAAAC  TGCCCACTTG  GCAGTACATC  AAGTGTATCA
241 TATGCCAAGT  ACGCCCCCTA  TTGACGTCAA  TGACGGTAAA  TGGCCCGCCT  GGCATTATGC
301 CCAGTACATG  ACCTTATGGG  ACTTTCCTAC  TTGGCAGTAC  ATCTACGTAT  TAGTCATCGC
361 TATTACCATG  GTGATGCGGT  TTTGGCAGTA  CATCAATGGG  CGTGGATAGC  GGTTTGACTC
421 ACGGGGATTT  CCAAGTCTCC  ACCCATTGA  CGTCAATGGG  AGTTTGTTTT  GGCACCAAAA
481 TCAACGGGAC  TTTCCAAAAT  GTCGTAACAA  CTCCGCCCCA  TTGACGCAAA  TGGGCGGTAG
541 GCGTGTACGG  TGGGAGGTCT  ATATAAGCAG  AGCTCGTTTA  GTGAACCGTC  AGATCGCCTG
601 GAGACGCCAT  CCACGCTGTT  TTGACCTCCA  TAGAAGACAC  CGGGACCGAT  CCAGCCTCCG
661 GACTCTAGCC  TAGGCCCGCG  AGCGGATAAC  AATTTTCACAC  AGGAAACAGC  TATGACCATT
721 AGGCCTTTGC  AAAAAGCTAT  TTAGGTGACA  CTATAGAAGG  TACGCCTGCA  GGTACCGGAT
781 CACAAGTTTG  TACAAAAAAG  CTGAACGAGA  AACGTAAAAT  GATATAAATA  TCAATATATT
841 AAATTAGATT  TTGCATAAAA  AACAGACTAC  ATAATACTGT  AAAACACAAC  ATATCCAGTC
901 ACTATGGCGG  CCGCATTAGG  CACCCAGGC  TTTACACTTT  ATGCTTCCGG  CTCGTATAAT
961 GTGTGGATTT  TGAGTTAGGA  TCCGTCGAGA  TTTTCAGGAG  CTAAGGAAGC  TAAAATGGAG
1021 AAAAAAATCA  CTGGATATAC  CACCGTTGAT  ATATCCCAAT  GGCAATCGTAA  AGAACATTTT
1081 GAGGCATTTT  AGTCAGTTGC  TCAATGTACC  TATAACCAGA  CCGTTCAGCT  GGATATTACG
1141 GCCTTTTAA  AGACCGTAAA  GAAAAATAAG  CACAAGTTTT  ATCCGGCCTT  TATTACATT
1201 CTTGCCCGCC  TGATGAATGC  TCATCCGGAA  TTCCGTATGG  CAATGAAAGA  CCGTGAGCTG
1261 GTGATATGGG  ATAGTGTTCA  CCCTTGTTAC  ACCGTTTTCC  ATGAGCAAAC  TGAAACGTTT
1321 TCATCGCTCT  GGAGTGAATA  CCACGACGAT  TTCCGGCAGT  TTCTACACAT  ATATTGCGAA
1381 GATGTGGCGT  GTTACGGTGA  AAACCTGGCC  TATTTCCCTA  AAGGGTTTAT  TGAGAATATG
1441 TTTTTCGTCT  CAGCCAATCC  CTGGGTGAGT  TTCACCAATT  TTGATTTAAA  CGTGGCCAAAT
1501 ATGGACAAC  TCTTCGCCCC  CGTTTTTACC  ATGGGCAAAT  ATTATACGCA  AGGCGACAAG
1561 GTGCTGATGC  CGCTGGCGAT  TCAGGTTTCT  CATGCCGTCT  GTGATGGCTT  CCATGTCCGC
1621 AGAATGCTTA  ATGAATTACA  ACAGTACTGC  GATGAGTGGC  AGGGCGGGGC  GTAAACGCGT
1681 GGATCCGGCT  TACTAAAAGC  CAGATAACAG  TATGCGTATT  TGC GCGCTGA  TTTTTCGGGT
1741 ATAAGAATAT  ATACTGATAT  GTATACCCGA  AGTATGTCAA  AAAGAGGTGT  GCTATGAAGC
1801 AGCGTATTAC  AGTGACAGTT  GACAGCGACA  GCTATCAGTT  GCTCAAGGCA  TATATGATGT
1861 CAATATCTCC  GGTCTGGTAA  GCACAACCAT  GCAGAATGAA  GCCCGTCGTC  TGCGTGCCGA
1921 ACGCTGGAAA  GCGGAAAATC  AGGAAGGGAT  GGCTGAGGTC  GCCCGTTTAA  TTGAAATGAA
1981 CGGCTCTTTT  GCTGACGAGA  ACAGGGACTG  GTGAAATGCA  GTTTAAGGTT  TACACCTATA
2041 AAAGAGAGAG  CCGTTATCGT  CTGTTTGTGG  ATGTACAGAG  TGATATTATT  GACACGCCCC
2101 GGCACGGGAT  GGTGATCCCC  CTGGCCAGTG  CACGTCTGCT  GTCAGATAAA  GTCTCCCGTG
2161 AACTTTACCC  GGTGGTGCAT  ATCGGGGATG  AAAGCTGGCG  CATGATGACC  ACCGATATGG
2221 CCAGTGTGCC  GGTCTCCGTT  ATCGGGGAAG  AAGTGGCTGA  TCTCAGCCAC  CGCGAAAATG
2281 ACATCAAAAA  CGCCATTAA  CTGATGTTCT  GGGGAATATA  AATGTCAGGC  TCCCTTATAC
2341 ACAGCCAGTC  TGCAGGTCCA  CCATAGTGAC  TGGATATGTT  GTGTTTACA  GTATTATGTA
2401 GTCTGTTTTT  TATGCAAAAT  CTAATTTAAT  ATATTGATAT  TTATATCATT  TTACGTTTCT
2461 CGTTCAGCTT  TCTTGTAACA  AGTGGTGATC  GCGTGCATGC  GACGTCATAG  CTCTCTCCCT
2521 ATAGTGAGTC  GTATTATAAG  CTAGGCACTG  GCCGTCGTTT  TACAACGTCG  TGACTGGGAA-

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2581 AACTGCTAGC TTGGGATCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC ATAATTGGAC  
2641 AACTACCTA CAGAGATTTA AAGCTCTAAG GTAAATATAA AATTTTTAAG TGTATAATGT  
2701 GTTAAACTAG CTGCATATGC TTGCTGCTTG AGAGTTTTCG TTAGTGAGTA TGATTATGA  
2761 AAATATTATA CACAGGAGCT AGTGATTCTA ATTGTTTGTG TATTTTAGAT TCACAGTCCC  
2821 AAGGCTCATT TCAGGCCCTT CAGTCCTCAC AGTCTGTTCA TGATCATAAT CAGCCATACC  
2881 ACATTTGTAG AGGTTTTACT TGCTTTAAAA AACCTCCAC ACCTCCCCCT GAACCTGAAA  
2941 CATAAATGA ATGCAATTGT TGTGTTAAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA  
3001 TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTCTACTGCA TTCTAGTTGT  
3061 GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCCTGC ATTAATGAAT  
3121 CGGCCAACGC GCGGGGAGAG GCGGTTTTCG TATTGGCTGG CGTAATAGCG AAGAGGCCCCG  
3181 CACCGATCGC CCTTCCCAAC AGTTGCGCAG CCTGAATGGC GAATGGGACG CGCCCTGTAG  
3241 CGGCGCATTG AGCGCGCGCG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG  
3301 CGCCCTAGCG CCCGCTCCTT TCGCTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGCGCTT  
3361 TCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC CGATTTAGTG CTTTACGGCA  
3421 CCTCGACCCC AAAAACTTG ATTAGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA  
3481 GACGGTTTTT CGCCCTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCGA  
3541 AACTGGAACA AACTCAACC CTATCTCGGT CTATTCTTTT GATTTATAAG GGATTTTGCC  
3601 GATTTGCGCC TATTGGTTAA AAAATGAGCT GATTTAACA AAATTTAACG CGAATTTTAA  
3661 CAAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC  
3721 TATTTGTTTA TTTTCTAAA TACATTCAAA TATGTATCCG CTCATGCCAG GTCTTGGAAGT  
3781 GGTGAGAACG GCTTGCTCGG CAGCTTCGAT GTGTGCTGGA GGGAGAATAA AGGTCTAAGA  
3841 TGTGCGATAG AGGGAAGTCG CATTGAATTA TGTGCTGTGT AGGGATCGCT GGTATCAAA  
3901 ATGTGTGCCC ACCCCTGGCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA  
3961 AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCTTTT TTGCGGCATT  
4021 TTGCCTTCCT GTTTTGTCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA  
4081 GTTGGGTGCA CGAGTGGGT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG  
4141 TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC  
4201 GCTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA  
4261 GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT  
4321 AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT  
4381 GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT  
4441 AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA  
4501 CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGGCAGAA CTATTAAGTG GCGAACTACT  
4561 TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAAGGACC  
4621 ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA  
4681 GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT  
4741 AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA  
4801 GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT  
4861 TTAGATTGAT TTAAACTTTC ATTTTAAAT TAAAAGGATC TAGGTGAAGA TCCTTTTGA  
4921 TAATCTCATG CCATAACTTC GTATAATGTA TGCTATACGA AGTTATGGCA TGACCAAAAT  
4981 CCCTTAACGT GAGTTTTCTG TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC  
5041 TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT  
5101 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTCCGA AGGTAACCTGG  
5161 CTTACGAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA  
5221 CTTCAAGAAC TCTGTAGCAC CGCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC  
5281 TGCTGCCAGT GCGGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA  
5341 TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC  
5401 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA  
5461 AGGGAGAAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCAGGAG  
5521 GGAGCTTCCA GGGGGAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG  
5581 ACTTGAGCGT CGATTTTGTG GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG  
5641 CAACGCGGCC TTTTACGGT TCTGCGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC  
5701 TGCGTTATCC CCTGATTCTG TGATAAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC  
5761 TCGCCGACG CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC  
5821 AATACGCAAA CCGCCTCTCC CCGCGGTTG GCCGATTCAT TAATGCAGAG CTTGCAATTC  
5881 GCGCGTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT  
5941 ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTT CCCGAAAAGT  
6001 GCCACCTGAC GTCTAAGAAA CCATT

Figure 28A: pDEST8 Polyhedron Promoter, Baculovirus Transfer Plasmid

1 *AceI*  
 cgt|ata ctc cgg aat att aat aga tca tgg aga taa tta aaa tga taa cca  
 gca tat gag gcc tta taa tta tct agt acc tct att aat ttt act att ggt  
 52 *→ mRNA (polyhedrin)*  
 tet cgc aaa taa ata agt att tta ctg ttt tgg taa cag ttt tgt aat aaa  
 aga gcg ttt att tat tca taa aat gac aaa agc att gtc aaa aca tta ttt  
 103  
 aaa acc tat aaa tat tcc gga tta ttc ata ccg tcc cac cat cgg gcg cgg  
 ttt tgg ata ttt ata agg cct aat aag tat ggc agg gtg gta gcc cgc gcc  
 154 *Bam* *Int* *KR1*  
 atc atc aca agt tgg tag aaa aaa gct gaa cga gaa aag taa tat gat ata  
 tag tag tgt tca aac atg ttt ttc cga ctt gct ctt tgc att tta cta tat



60/240

## pDEST8 6526 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
23..152		Ppolh
284..160		attR1
534..1193		CmR
1313..1397		inactivated ccdA
1535..1840		ccdB
1881..2005		attR2
2766..3146		f1
3240..4090		ampR
4289..4869		ori
5564..6496		genR

1	CGTATACTCC	GGAATATTAA	TAGATCATGG	AGATAATTAA	AATGATAACC	ATCTCGCAAA
61	TAAATAAGTA	TTTTACTGTT	TTCGTAACAG	TTTTGTAAATA	AAAAAACCTA	TAAATATTCC
121	GGATTATTCA	TACCGTCCCA	CCATCGGGCG	CGGATCATCA	CAAGTTTGTA	CAAAAAAGCT
181	GAACGAGAAA	CGTAAATGA	TATAAATATC	AATATATTAA	ATTAGATTTT	GCATAAAAAA
241	CAGACTACAT	AATACTGTAA	AACACAACAT	ATCCAGTCAC	TATGGCGGCC	GCTAAGTTGG
301	CAGCATCACC	CGACGCACCT	TGCGCCGAAT	AAATACCTGT	GACGGAAGAT	CACCTTCGCAG
361	AATAAATAAA	TCCTGGTGTC	CCTGTTGATA	CCGGGAAGCC	CTGGGCCAAC	TTTTGGCGAA
421	AATGAGACGT	TGATCGGCAC	GTAAGAGGTT	CCAACCTTCA	CCATAATGAA	ATAAGATCAC
481	TACCGGGCGT	ATTTTTTGAG	TTATCGAGAT	TTTCAGGAGC	TAAGGAAGCT	AAAATGGAGA
541	AAAAAATCAC	TGGATATACC	ACCGTTGATA	TATCCCAATG	GCATCGTAAA	GAACATTTTG
601	AGGCATTTCA	GTCAGTTGCT	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG	GATATTACGG
661	CCTTTTTTAAA	GACCGTAAAG	AAAAATAAGC	ACAAGTTTTA	TCCGGCCTTT	ATTCACATTC
721	TTGCCCGCCT	GATGAATGCT	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC	GGTGAGCTGG
781	TGATATGGGA	TAGTGTTTAC	CCTTGTTACA	CCGTTTTCCA	TGAGCAAACT	GAAACGTTTT
841	CATCGCTCTG	GAGTGAATAC	CACGACGATT	TCCGGCAGTT	TCTACACATA	TATTCGCAAG
901	ATGTGGCGTG	TTACGGTGAA	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT	GAGAATATGT
961	TTTTCGTCTC	AGCCAATCCC	TGGGTGAGTT	TCACCAGTTT	TGATTTAAAC	GTGGCCAATA
1021	TGGACAACCT	CTTCGCCCCC	GTTTTTCACCA	TGGGCAAATA	TTATACGCCAA	GGCGACAAGG
1081	TGCTGATGCC	GCTGGCGATT	CAGGTTTCATC	ATGCCGCTCTG	TGATGGCTTC	CATGTCGGCA
1141	GAATGCTTAA	TGAATTACAA	CAGTACTGCG	ATGAGTGGCA	GGGCGGGGCG	TAAACGCGTG
1201	GATCCGGCTT	ACTAAAAGCC	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT	TTTTGCGGTA
1261	TAAGAATATA	TACTGATATG	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG	CTATGAAGCA
1321	GCGTATTACA	GTGACAGTTG	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT	ATATGATGTC
1381	AATATCTCCG	GTCTGGTAAG	CACAACCATG	CAGAATGAAG	CCCGTCGTCT	GCGTGCCGAA
1441	CGCTGGAAG	CGGAAAATCA	GGAAGGGATG	GCTGAGGTG	CCCGGTTTTAT	TGAAATGAAC
1501	GGCTCTTTTG	CTGACGAGAA	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT	ACACCTATAA
1561	AAGAGAGAGC	CGTTATCGTC	TGTTTGTGGA	TGTACAGAGT	GATATTATTG	ACACGCCCGG
1621	GCGACGGATG	GTGATCCCCC	TGGCCAGTGC	ACGTCTGCTG	TCAGATAAAG	TCTCCCGTGA
1681	ACTTTACCCG	GTGGTGCCATA	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA	CCGATATGGC
1741	CAGTGTGCCG	GTCTCCGTTA	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC	GCGAAAATGA
1801	CATCAAAAAC	GCCATTAACC	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT	CCCTTATACA
1861	CAGCCAGTCT	GCAGGTCGAC	CATAGTGACT	GGATATGTTG	TGTTTTACAG	TATTATGTAG
1921	TCTGTTTTTT	ATGCAAAATC	TAATTTAATA	TATTGATATT	TATATCATTT	TACGTTTCTC
1981	GTTTCTGCTT	CTTGTACAAA	GTGGTGATAG	CTTGTGCGAGA	AGTACTAGAG	GATCATAATC
2041	AGCCATACCA	CATTTGTAGA	GGTTTTACTT	GCTTTAAAAA	ACCTCCACAC	CCTCCCCCTG
2101	AACCTGAAAC	ATAAAATGAA	TGCAATTGTT	GTTGTAACT	TGTTTATTGC	AGCTTATAAT
2161	GGTTACAAAT	AAAGCAATAG	CATCACAAT	TTCACAAATA	AAGCATTTTT	TTCACATGCAT
2221	TCTAGTTGTG	GTTTGTCCTA	ACTCATCAAT	GTATCTTATC	ATGCTGGGAT	CTGATCACTG
2281	CTTAGGCTTA	GGAGATCCGA	ACCAGATAAG	TGAAATCTAG	TTCCAAACTA	TTTTGTCTATT
2341	TTTAATTTTC	GTATTAGCTT	ACGACGCTAC	ACCCAGTTCC	CATCTATTTT	GTCATCTTTC
2401	CCTAAATAAT	CCTTAAAAAC	TCCATTTCCA	CCCCTCCCAG	TTCCCAACTA	TTTTGTCCGC
2461	CCACAGCGGG	GCATTTTCT	TCCTGTTATG	TTTTTAATCA	AACATCCTGC	CAACTCCATG
2521	TGACAAACCG	TCATCTTCGG	CTACTTTTTT	TCTGTACACAG	AATGAAAATT	TTTCTGTCAT

FIGURE 28B



2581 CTCTTCGTTA TTAATGTTTG TAATTGACTG AATATCAACG CTTATTTGCA GCCTGAATGG  
2641 CGAATGGACG CGCCCTGTAG CGGCGCATT AAGCGGCGG GTGTGGTGGT TACGCGCAGC  
2701 GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT  
2761 CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC  
2821 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTAGGGTGA TGTTTCACGT  
2881 AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA CGTTGGAGTC CACGTTCTTT  
2941 AATAGTGGAC TCTTGTTCCA AACTGGAACA AACTCAACC CTATCTCGGT CTATTCTTTT  
3001 GATTTATAAG GGATTTTGCC GATTTCCGGC TATTGGTTAA AAAATGAGCT GATTTAACAA  
3061 AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG  
3121 GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTCTTAAA TACATTCAAA TATGTATCCG  
3181 CTCATGAGAC AATAACCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT  
3241 ATTCAACATT TCCGTGTGCG CCTTATTCCC TTTTGTGCGG CATTGTGCCT TCCTGTTTTT  
3301 GCTCACCCAG AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG  
3361 GGTTACATCG AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCCAGAGAA  
3421 CGTTTTCCAA TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT  
3481 GACGCCGGGC AAGAGCAACT CGGTGCGCGC ATACACTATT CTCAGAATGA CTGTTTGAG  
3541 TACTCACCAG TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT  
3601 GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA  
3661 CCGAAGGAGC TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACGCG CTTGATCGT  
3721 TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA  
3781 GCAATGGCAA CAACGTTGCG CAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG  
3841 CAACAATTAA TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCCTTCT GCGCTCGGCC  
3901 CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT  
3961 ATCATTGCAG CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGAGC  
4021 GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCTCACTG  
4081 ATTAAGCATT GGTAACGTG AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA  
4141 CTTCAATTTT AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA  
4201 ATCCCTTAAC GTGAGTTTTT GTTCCACTGA CCGTCAGACC CCGTAGAAAA GATCAAAGGA  
4261 TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAAACAA AAAACCACCG  
4321 CTACCAGCGG TGGTTGTTTT GCCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAAC  
4381 GGCTTCAGCA GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC  
4441 CACTTCAAGA ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG  
4501 GCTGCTGCCA GTGGCGATAA GTCGTGTCTT ACCGGGTGG ACTCAAGACG ATAGTTACCG  
4561 GATAAGGCCG AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCGAG CTTGGAGCGA  
4621 ACGACCTACA CCGAACTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC  
4681 GAAGGGAGAA AGGCGGACAG GTATCCGTA AGCGGCAGGG TCGGAACAGG AGAGCGCAGC  
4741 AGGGAGCTTC CAGGGGAAAA CGCCTGGTAT CTTTATAGTC CTGTGCGGTT TCGCCACCTC  
4801 TGACTTGAGC GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC  
4861 AGCAACGCGG CTTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTGTCTCA CATGTTCTTT  
4921 CCTGCGTTAT CCCCTGATTC TGTGGATAAC CGTATTACCG CTTTGTAGTG AGCTGATACC  
4981 GCTCGCCGCA GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC  
5041 CTGATGCGGT ATTTTCTCCT TACGCATCTG TCGGTATTTT CACACCGCAG ACCAGCCGCG  
5101 TAACCTGGCA AAATCGGTTA CGGTTGAGTA ATAAATGGAT GCCCTGCGTA AGCGGGTGTG  
5161 GGCGGACAAT AAAGTCTTAA ACTGAACAAA ATAGATCTAA ACTATGACAA TAAAGTCTTA  
5221 AACTAGACAG AATAGTTGTA AACTGAAATC AGTCCAGTTA TGCTGTGAAA AAGCATACTG  
5281 GACTTTTGTT ATGGCTAAAG CAACTCTTC ATTTTCTGAA GTGCAAATTG CCCGTCGTAT  
5341 TAAAGAGGGG CGTGGCCAAG GGCATGGTAA AGACTATATT CGCGGCGTTG TGACAATTTA  
5401 CCGAACAAC CCGCGGCCGG GAAGCCGATC TCGGCTTGAA CGAATTGTTA GGTGGCGGTA  
5461 CTTGGGTCGA TATCAAAGTG CATCACTTCT TCCCGTATGC CCAACTTTGT ATAGAGAGCC  
5521 ACTGCGGGAT CGTCACCGTA ATCTGCTTGC ACGTAGATCA CATAAGCACC AAGCGCGTTG  
5581 GCCTCATGCT TGAGGAGATT GATGAGCGCG GTGGCAATGC CCTGCCTCCG GTGCTCGCCG  
5641 GAGACTGCGA GATCATAGAT ATAGATCTCA CTACGCGGCT GCTCAAACCT GGGCAGAACG  
5701 TAAGCCGCGA GAGCGCCAAC AACCGCTTCT TGGTCGAAGG CAGCAAGCGC GATGAATGTC  
5761 TTAACACGGA GCAAGTTCCC GAGGTAATCG GAGTCCGCT GATGTTGGGA GTAGGTGGCT  
5821 ACGTCTCCGA ACTCACGACC GAAAAGATCA AGAGCAGCCC GCATGGATTT GACTTGGTCA  
5881 GGGCCGAGCC TACATGTGCG AATGATGCCC ATACTTGAGC CACCTAACTT TGTTTTAGGG  
5941 CGACTGCCCT GCTGCGTAAC ATCGTTGCTG CTGCGTAACA TCGTTGCTGC TCCATAACAT  
6001 CAAACATCGA CCCACGGCGT AACGCGCTTG CTGCTTGGAT GCCCGAGGCA TAGACTGTAC

FIGURE 28C

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6061 AAAAAACAG TCATAACAAG CCATGAAAAC CGCCACTGCG CCGTTACCAC CGCTGCGTTC  
6121 GGTCAAGGTT CTGGACCAGT TGGGTGAGCG CATACGCTAC TTGCATTACA GTTTACGAAC  
6181 CGAACAGGCT TATGTCAACT GGGTTCGTGC CTTTCATCCGT TTCCACGGTG TGGTCAACCC  
6241 GGCAACCTTG GGCAGCAGCG AAGTCGAGGC ATTTCTGTCC TGGCTGGCGA ACGAGCGCAA  
6301 GGTTTCGGTC TCCACGCATC GTCAGGCATT GCGGGCCTTG CTGTTCTTCT ACGGCAAGGT  
6361 GCTGTGCACG GATCTGCCCT GGCTTCAGGA GATCGGAAGA CCTCGGCCGT CGCGGCGCTT  
6421 GCCGGTGGTG CTGACCCCGG ATGAAGTGGT TCGCATCCTC GGTTTTCTGG AAGGCGAGCA  
6481 TCGTTTGTTT GCCCAGGACT CTAGCTATAG TTCTAGTGGT TGGCTA

FIGURE 28D

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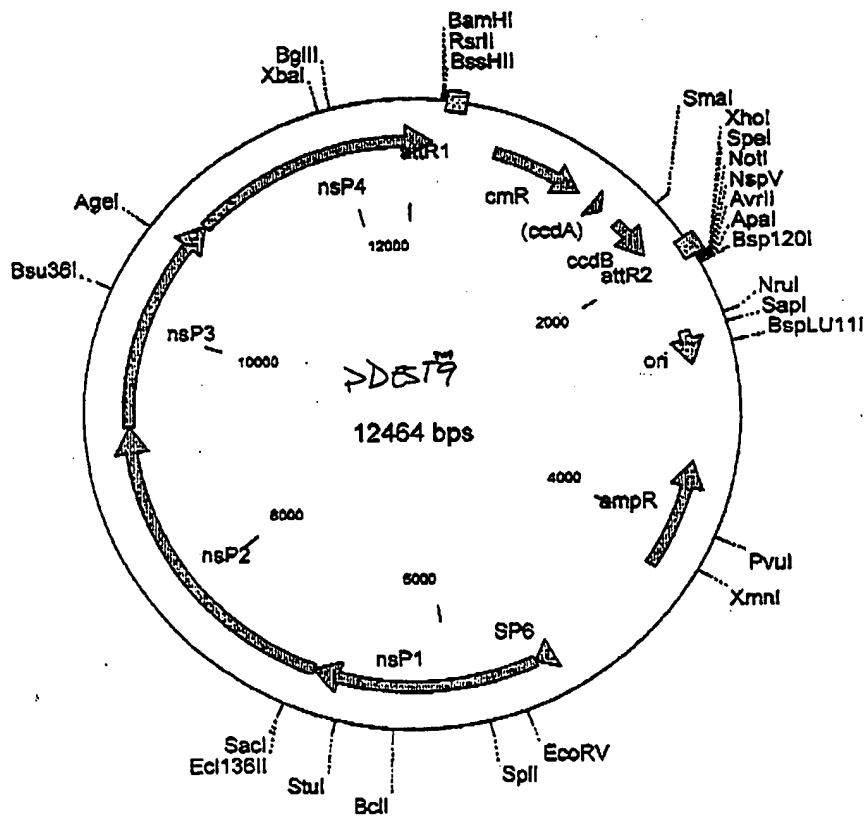
Figure 29A: pDEST9

## Semliki Forest Virus vector

103 ttg gcg agg gac att aag gcg ttt aag aaa ttg aga gga cct gtt ata ~~cac~~  
 aac cgc tcc ctg taa ttc cgc aaa ttc ttt aac tct cct gga caa tat ~~gtg~~  
 243 ~~prokdr~~ → 245 ~~RNA~~ ~~Bam~~

154 ~~ctc tac ggc ggt cct aga ttg gtc cgt taa tac aca gaa ttc tga ttg gat~~  
~~gag atg ccg cca gga tct aac cac gca att atg tgt ctt aag act aac cta~~

205 ~~ccc ggt ccg aag cgc gct ttc cca tca~~ ~~aca agt tgg tac aac aag gct gaa~~  
~~ggg cca ggc ttc gcg cga aag ggt agt~~ ~~tgt tca aac atg ttt tta cga ctt~~



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## pDEST9 12464 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
355..232		attR1
605..1264		CmR
1384..1468		inactivated ccdA
1606..1911		ccdB
1952..2078		attR2
2532..2782		ori
3482..4282		ampR
5232..5365		SP6 promoter
5365..6965		nsP1:non-structural protein 1
6965..9265		nsP2:non-structural protein 2
9265..10865		nsP3:non-structural protein 3
10865..161		nsP4:non-structural protein 4

1	AGCAAGTGGT	TCCGGACAGG	CTTGGGGGCC	GAAGTGGAGG	TGGCACTAAC	ATCTAGGTAT
61	GAGGTAGAGG	GCTGCAAAAG	TATCCTCATA	GCCATGGCCA	CCTTGGCGAG	GGACATTAAG
121	GCGTTTAAGA	AATTGAGAGG	ACCTGTTATA	CACCTCTACG	GCGGTCCTAG	ATTGGTGGT
181	TAATACACAG	AATTCTGATT	GGATCCCGGT	CCGAAGCGCG	CTTCCCACATC	ACAAGTTTGT
241	ACAAAAAAGC	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA	AATTAGATTT
301	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA	CTATGGCGGC
361	CGCTAAGTTG	GCAGCATCAC	CCGACGCACT	TTGCGCCGAA	TAAATACCTG	TGACGGAAGA
421	TCACCTCGCA	GAATAAATA	ATCCTGGTGT	CCCTGTTGAT	ACCGGGAAGC	CCTGGGCCAA
481	CTTTTGGCGA	AAATGAGACG	TTGATCGGCA	CGTAAGAGGT	TCCAACCTTC	ACCATAATGA
541	AATAAGATCA	CTACCGGGCG	TATTTTTTGA	GTTATCGAGA	TTTTCAGGAG	CTAAGGAAGC
601	TAAATGGAG	AAAAAATCA	CTGGATATAC	CACCGTTGAT	ATATCCCAAT	GGCATCGTAA
661	AGAACATTTT	GAGGCATTTC	AGTCAGTTGC	TCAATGTACC	TATAACCAGA	CCGTTACAGT
721	GGATATTACG	GCCTTTTTTA	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT	ATCCGGCCTT
781	TATTCACATT	CTTGCCCGCC	TGATGAATGC	TCATCCGGAA	TTCCGTATGG	CAATGAAAGA
841	CGGTGAGCTG	GTGATATGGG	ATAGTGTTC	CCCTTGTTAC	ACCGTTTTTC	ATGAGCAAAC
901	TGAAACGTTT	TCATCGCTCT	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT	TTCTACACAT
961	ATATTCGCAA	GATGTGGCGT	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA	AAGGGTTTTAT
1021	TGAGAATATG	TTTTTCGTCT	CAGCCAATCC	CTGGGTGAGT	TTCAACAGTT	TTGATTTTAA
1081	CGTGGCCAAT	ATGGACAAC	TCTTCGCCCC	CGTTTTCAAC	ATGGGCAAAT	ATTATACGCA
1141	AGGCGACAAG	GTGCTGATGC	CGCTGGCGAT	TCAGGTTTCAT	CATGCCGTCT	GTGATGGCTT
1201	CCATGTCCGC	AGAATGCTTA	ATGAATTACA	ACAGTACTGC	GATGAGTGGC	AGGGCGGGGC
1261	GTAAGATCT	GGATCCGGCT	TACTAAAAGC	CAGATAACAG	TATGCGTATT	TGCGCGCTGA
1321	TTTTTGCGGT	ATAAGAATAT	ATACTGATAT	GTATACCCGA	AGTATGTCAA	AAAGAGGTGT
1381	GCTATGAAGC	AGCGTATTAC	AGTGACAGTT	GACAGCGACA	GCTATCAGTT	GCTCAAGGCA
1441	TATATGATGT	CAATATCTCC	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA	GCCCCGTCGTC
1501	TGCGTGCCGA	ACGCTGGAAA	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC	GCCCCGTTTA
1561	TTGAAATGAA	CGGCTCTTTT	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA	GTTTAAGGTT
1621	TACACCTATA	AAAGAGAGAG	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG	TGATATTATT
1681	GACACGCCCG	GCGGACGGAT	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT	GTGAGATAAA
1741	GTCTCCCGTG	AACCTTACCC	GGTGGTGATC	ATCGGGGATG	AAAGCTGGCG	CATGATGACC
1801	ACCGATATGG	CCAGTGTGCC	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA	TCTCAGCCAC
1861	CGCGAAAATG	ACATCAAAAA	CGCCATTAAAC	CTGATGTTCT	GGGGAATATA	AATGTCAGGC
1921	TCCCTTATAC	ACAGCCAGTC	TGCAGGTGCA	CCATAGTGAC	TGGATATGTT	GTGTTTTTACA
1981	GTATTATGTA	GTCTGTTTTT	TATGCAAAAG	TGCTAATTTA	ATATATTGAT	ATTTATATCA
2041	TTTTACGTTT	CTCGTTACGC	TTTCTTGATC	AAAGTGGTGA	TGGGAACCTCG	AGTTCACCTAG
2101	TCGATCCCGC	GGCCGCTTTC	GAACCTAGGC	AAGCATGCGG	GCCCAGTGGG	TAATTAATTG
2161	AATTACATCC	CTACGCAAAC	GTTTTACGGC	CGCCGGTGGC	GCCCAGCGCC	GCGCCCGCGT
2221	CCTTGGCCGT	TGCAGGCCAC	TCCGGTGGCT	CCCGTCGTCC	CCGACTTCCA	GGCCAGCAG
2281	ATGCAGCAAC	TCATCAGCGC	CGTAAATGCG	CTGACAATGA	GACAGAACGC	AATTGCTCCT
2341	GCTAGGAGCT	TAATTCGACG	AATAATTGGA	TTTTTATTTT	ATTTTGCAAT	TGTTTTTTAA
2401	TATTTCCAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA

FIGURE 29B

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2461 AAAAAAAAAA AAAAAAACTA GAAATCGCGA TTTCTAGTCT GCATTAATGA ATCGGCCAAC  
2521 GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC  
2581 TGCGCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT  
2641 TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG  
2701 CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCTGACG  
2761 AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT  
2821 ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TCGCTCTCC TGTTCGACC CTGCCGCTTA  
2881 CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTCTCAA TGCTCGCGCT  
2941 GTAGGTATCT CAGTTCGGTG TAGGTCGTTT GCTCCAAGCT GGGCTGTGTG CACGAACCCC  
3001 CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACATATC TCTTGAGTCC AACCCGGTAA  
3061 GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG  
3121 TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG  
3181 TATTTGGTAT CTGCGCTETG CTGAAGCCAG TTACCTTCGG AAAAAAGATT GGTAGCTCTT  
3241 GATCCGGCAA ACAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA  
3301 CCGGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TGCTCGCTC  
3361 AGTGAACGA AAACCTCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA  
3421 CCTAGATCCT TTAAATTA AAATGAAGTT TTAATCAAT CTAAAGTATA TATGAGTAAA  
3481 CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT  
3541 TTCGTTTATC CATAGTTGCC TGAATCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT  
3601 TACCATCTGG CCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT  
3661 TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCTT GCAACTTTAT  
3721 CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGAAGCTAG AGTAAAGTAG TCGCCAGTTA  
3781 ATAGTTTGGC CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTACGCG TCGTCGTTTG  
3841 GTATGGCTTC ATTCAGCTCC GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCATGT  
3901 TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTGAGAAGT AAGTTGGCCG  
3961 CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG  
4021 TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC  
4081 GCGGACCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGGCCCA CATAGCAGAA  
4141 CTTTAAAGT GCTCATCATT GGAAAACGTT CTTGCGGGCG AAAACTCTCA AGGATCTTAC  
4201 CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT  
4261 TTACTTTCAC CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG  
4321 GAATAAGGGC GACACGAAA TGTGAATAC TCATACTCTT CCTTTTCAA TATTATTGAA  
4381 GCATTTATCA GGGTTATTGT CTGATGAGCG GATACATATT TGAATGTATT TAGAAAAATA  
4441 AACAAATAGG GGTTCCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA  
4501 TTATTATCAT GACATTAACC TATAAAAATA GCGGTATCAC GAGGCCCTTT CGTCTCGCGC  
4561 GTTTCGGTGA TGACGGTGAA AACCTCTGAC ACATGCAGCT CCCGAGACG GTCACGCTT  
4621 CTGTCTAAGC GGATGCCGGG AGCAGACAAG CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG  
4681 GGTGTCGGGG CTGGCTTAAC TATGCGGCAT CAGAGCAGAT TGTACTGAGA GTGCACCATA  
4741 TCGACGCTCT CCCTTATGCG ACTCCTGCAT TAGGAAGCAG CCCAGTACTA GGTGAGGCC  
4801 GTTGAGCACC GCCGCCGCAA GGAATGGTGC ATGCAAGGAG ATGGCGCCCA ACAGTCCCCC  
4861 GGCCACGGGG CCTGCCACCA TACCCACGCC GAAACAAGCG CTCATGAGCC CGAAGTGGCG  
4921 AGCCCGATCT TCCCCATCGG TGATGTCGGC GATATAGGCG CCAGCAACCG CACCTGTGGC  
4981 GCCGGTGATG CCGGCCACGA TGCGTCCGGC GTAGAGGATC TGGCTAGCGA TGACCTGTCT  
5041 GATTGGTTCG CTGACCATTT CCGGGGTGCG GAACGGCGTT ACCAGAAACT CAGAAGGTTT  
5101 GTCCAACCAA ACCGACTCTG ACGGCAGTTT ACGAGAGAGA TGATAGGGTC TGCTTCAGTA  
5161 AGCCAGATGC TACACAATTA GGCTTGTA CA TATTGTCGTT AGAACCGGGC TACAATTAAT  
5221 ACATAACCTT ATGTATCATA CACATACGAT TTAGGTGACA CTATAGATGG CGGATGTGTG  
5281 ACATACACGA CGCCAAAAGA TTTGTTTCCA GCTCCTGCCA CCTCCGCTAC GCGAGAGATT  
5341 AACCACCCAC GATGGCCGCC AAAGTGCATG TTGATATTGA GGCTGACAGC CCATTTCATCA  
5401 AGTCTTTGCA GAAGGCATTT CCGTCGTTCC AGGTGGAGTC ATTGCAGGTC ACACCAAATG  
5461 ACCATGCAAA TGCCAGAGCA TTTTCGCACC TGGCTACCAA ATTGATCGAG CAGGAGCATG  
5521 ACAAAGACAC ACTCATCTTG GATATCGGCA GTGCGCCTTC CAGGAGAATG ATGTCTACGC  
5581 ACAAATACCA CTGCGTATGC CCTATGCGCA GCGCAGAAGA CCCCAGAAAG CTCGATAGCT  
5641 ACGCAAAGAA ACTGGCAGCG GCCTCCGGGA AGGTGCTGGA TAGAGAGATC GCAGGAAAAA  
5701 TCACCGACCT GCAGACCGTC ATGGCTACGC CAGACGCTGA ATCTCTTACC TTTTGCTGTC  
5761 ATACAGACGT CACGTGTCGT ACGGCAGCCG AAGTGGCCGT ATACCAGGAC GTGTATGCTG  
5821 TACATGCACC AACATCGCTG TACCATCAGG CGATGAAAGG TGTGAGAAGC GCGTATTGGA  
5881 TTGGGTTTGA CACCACCCCG TTTATGTTTG ACGCGCTAGC AGGCGCGTAT CCAACCTACG-

FIGURE 29C

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5941 CCACAAACTG GGCCGACGAG CAGGTGTTAC AGGCCAGGAA CATAGGACTG TGTGCA3CAT  
6001 CCTTGACTGA GGGGAAGACTC GGCAAACTGT CCATTCTCCG CAAGAAGCAA TTGAAAACCTT  
6061 GCGACACAGT CATGTTCTCG GTAGGATCTA CATTGTACAC TGAGAGCAGA AAGCTACTGA  
6121 GGAGCTGGCA CTTACCCTCC GTATTCCACC TGAAAGGTAA ACAATCCTTT ACCTGTAGGT  
6181 GCGATACCAT CGTATCATGT GAAGGGTACG TAGTTAAGAA AATCACTATG TGCCCC3GCC  
6241 TGTACGGTAA AACGGTAGGG TACGCCGTGA CGTATCACGC GGAGGGATTG CTAGTGTGCA  
6301 AGACCACAGA CACTGTCAAA GGAGAAAGAG TCTCATTCCC TGTATGCACC TACGTCCCTT  
6361 CAACCATCTG TGATCAAATG ACTGGCATACT TAGCGACCGA CGTCACACCG GAGGACGCAC  
6421 AGAAGTTGTT AGTGGGATTG AATCAGAGGA TAGTTGTGAA CGGAAGAACA CAGCGAAACA  
6481 CTAACACGAT GAAGAACTAT CTGCTTCCGA TTGTGGCCGT CGCATTTAGC AAGTGG3CGA  
6541 GGGAAATACAA GGCAGACCTT GATGATGAAA AACCTCTGGG TGTCCGAGAG AGGTCACTTA  
6601 CTTGCTGCTG CTTGTGGGCA TTTAAAACGA GGAAGATGCA CACCATGTAC AAGAAACCAG  
6661 ACACCCAGAC AATAGTGAAG GTGCCCTCAG AGTTTAACTC GTTCGTCATC CCGAGCCTAT  
6721 GGTCTACAGG CCTCGCAATC CCAGTCAGAT CACGCATTAA GATGCTTTTG GCCAAGAAGA  
6781 CCAAGCGAGA GTTAATACCT GTTCTCGACG CGTCGTCAGC CAGGGATGCT GAACAASAGG  
6841 ACAGGAAACT ATACCATATT GCCGTTACG GAGCTGACTA ACCACCCCTC GTCCGAAACG  
6901 CGCCGGCGGA GACGGGAGTC GTCGACGTCG ACGTTGAAGA ACTAGAGTAT CACGCA3GTG  
6961 CAGGGGTCTG GGAACACCT CGCAGCGCGT TGAAAGTCAC CGCACAGCCG AACGAC3TAC  
7021 TACTAGGAAA TTACGTAGTT CTGTCCCCGC AGACCGTGCT CAAGAGCTCC AAGTTG3CCC  
7081 CCGTGCACCC TCTAGCAGAG CAGGTGAAAA TAATAACACA TAACGGGAGG GCCGG3CGTT  
7141 ACCAGGTCGA CGGATATGAC GGCAGGGTCC TACTACCATG TGGATCGGCC ATTCCG3TCC  
7201 CTGAGTTTCA GGCTTTGAGC GAGAGCGCCA CTATGGTGTA CAACGAAAGG GAGTTCT3TCA  
7261 ACAGGAAACT ATACCATATT GCCGTTACG GAGCTGACTA ACCACCCCTC GTCCGAAACG  
7321 ACGAGAAAGT CAGAGCTGAA AGAACTGACG CCGAGTACGT GTTCGACGTA GATAAAAAAT  
7381 GCTGCGTCAA GAGAGAGGAA GCGTCGGGTT TGGTGTGGT GGGAGAGCTA ACCAACCC3C  
7441 CGTTCATGA ATTGCGCTAC GAAGGCTGA AGATCAGGCC GTCGGCACCA TATAAGACTA  
7501 CAGTAGTAGG AGTCTTTGGG GTTCCGGGAT CAGGCAAGTC TGCTATTATT AAGAGC3TCG  
7561 TGACCAAACA CGATCTGGTC ACCAGCGGCA AGAAGGAGAA CTGCCAGGAA ATAGTTA3ACG  
7621 ACGTGAAGAA GCACCGCGGG AAGGGGACAA GTAGGGAAAA CAGTGA3CTCC  
7681 ACGGGTGTG TCGTGGCGTG GACATCTTAT ATGTGGACGA GGCTTTTCGCT TTGCTT3CCG  
7741 GTACTCTGCT GGCCCTAATT GCTCTTGTTA AACCTCGGAG CAAAGTGGTG TTATGC3GAG  
7801 ACCCAAGCA ATGCGGATTC TTCAATATGA TGCAGCTTAA GGTGA3CTTC  
7861 TCTGCACTGA AGTATGTCAT AAAAGTATAT CCAGACGTTG CACGCGTCCA GTCACG3CCA  
7921 TCGTGTCTAC GTTGCACTAC GGAGGCAAGA TGCGCACGAC CAACCCGTGC AACAA3CCCA  
7981 TAATCATAGA CACCACAGGA CAGACCAAGC CCAAGCCAGG AGACATCGTG TTAACAT3GCT  
8041 TCCGAGGCTG GGCAAAGCAG CTGCAGTTGG ACTACCGTGG ACACGAAGTC ATGAC3CAG  
8101 CAGCATCTCA GGGCCTCACC CGCAAAGGGG TATACGCCGT AAGGCAGAAG GTGAAT3AAA  
8161 ATCCCTTGTA TGCCCTGCG TCGGACACG TGAATGTACT GCTGACGCGC ACTGAG3ATA  
8221 GGCTGGTGTG GAAAACGCTG GCCGGCGATC CCTGGATTAA GGTCTAT3CA  
8281 AGGGTAACTT TACGGCCACA TTGGAAGAAT GGCAAGAAGA ACACGACAAA ATAATG3AAG  
8341 TGATTGAAGG ACCGGCTGCG CCTGTGGACG CGTTCAGAA CAAAGCGAAC GTGTGT3GGG  
8401 CGAAAAGCCT GGTGCCTGTC CTGGACACTG CCGGAATCAG ATTGACAGCA GAGGAGT3GA  
8461 GCACCATAAT TACAGCATTT AAGGAGGACA GAGCTTACTC TCCAGTGGTG GCCTTGA3TG  
8521 AAATTTGCAC CAAGTACTAT GGAGTTGACC TGGACAGTGG CCTGTTTTCT GCCCGA3AGG  
8581 TGTCCCTGTA TTACGAGAAC AACCCTGGG ATAACAGACC TGGTGAAGG ATGTAT3GAT  
8641 TCAATGCCGC AACAGCTGCC AGGCTGGAAG CTAGACATAC CTTCTGAAG GGGCAGT3GC  
8701 ATACGGGCAA GCAGGCAGTT ATCGCAGAAA GAAAAATCCA ACCGCTTTCT GTGCTG3ACA  
8761 ATGTAATTCC TATCAACCGC AGGCTGCCGC ACGCCCTGGT GGCTGAGTAC AAGACG3TTA  
8821 AAGGCAGTAG GGTGAGTGG CTGGTCAATA AAGTAAGAGG GTACCACGTC CTGCTG3TGA  
8881 GTGAGTACAA CCTGGCTTTG CCTCGACGCA GGGTCACTTG GTTGTACCG CTGAAT3TCA  
8941 CAGGCGCCGA TAGGTGCTAC GACCTAAGTT TAGGACTGGG GGCTGACGCC GGCAGT3TCG  
9001 ACTTGGTCTT TGTGAACATT CACACGGAAT TCAGAAATCCA CCACTACCAG CAGTGT3TCG  
9061 ACCACGCCAT GAAGCTGCAG ATGCTTGGGG GAGATGCGCT ACGACTGCTA AAACCC3GCG  
9121 GCATCTTGAT GAGAGCTTAC GGATACGCCG ATAAAATCAG CGAAGCCGTT GTTTCT3CT  
9181 TAAGCAGAAA GTTCTCGTCT GCAAGAGTGT TGCGCCCGGA TTGTGTCACC AGCAAT3ACAG  
9241 AAGTGTCTTT GCTGTTCTCC AACTTTGACA ACGGAAAGAG ACCCTCTACG CTACAC3AGA  
9301 TGAATACCAA GCTGAGTGCC GTGTATGCCG GAGAAGCCAT GCACACGGCC GGGTGT3CAC  
9361 CATCCTACAG AGTTAAGAGA GCAGACATAG CCACGTGCAC AGAAGCGGCT GTGTTA3ACG-

FIGURE 29A

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9421 CAGCTAACGC CCGTGGAAC TTAGGGGATG GCGTATGCAG GGCCGTGGCG AAGAAATGGC  
9481 CGTCAGCCTT TAAGGGAGCA GCAACACCAG TGGGCACAAT TAAAACAGTC ATGTGCGGCT  
9541 CGTACCCCGT CATCCACGCT GTAGCGCCTA ATTTCTCTGC CACGACTGAA GCGGAAGGGG  
9601 ACCGCGAATT GGCCGCTGTC TACCGGGCAG TGGCCGCCGA AGTAAACAGA CTGTCACTGA  
9661 GCAGCGTAGC CATCCCGCTG CTGTCCACAG GAGTGTTCAG CGGCGGAAGA GATAGGCTGC  
9721 AGCAATCCCT CAACCATCTA TTCACAGCAA TGGACGCCAC GGACGCTGAC GTGACCATCT  
9781 ACTGCAGAGA CAAAAGTTGG GAGAAGAAAA TCCAGGAAGC CATTGACATG AGGACGGCTG  
9841 TGGAGTTGCT CAATGATGAC GTGGAGCTGA CCACAGACTT GGTGAGAGTG CACCCGGACA  
9901 GGAAGCTGGT GGGTCGTAA GGTACAGTA CCACTGACGG GTCGCTGTAC TCGTACTTTG  
9961 AAGGTACGAA ATTCAACCAG GCTGCTATTG ATATGGCAGA GATACTGACG TTGTGGCCCA  
10021 GACTGCAAGA GGCAAACGAA CAGATATGCC TATACGCGCT GGGCGAAACA ATGGACAACA  
10081 TCAGATCCAA ATGTCCGGTG AACGATTCCG ATTCATCAAC ACCTCCCAGG ACAGTGCCCT  
10141 GCCTGTGCCG CTACGCAATG ACAGCAGAAC GGATCGCCCC CCTTAGGTCA CACCAAGTTA  
10201 AAAGCATGGT GGTTCGCTCA TCTTTTCCCC TCCCGAAATA CCATGTAGAT GGGGTGCAGA  
10261 AGGTAAAGTG CGAGAAGGTT CTCCTGTTTCG ACCCGACGGT ACCTTCAGTG GTTAGTCCGC  
10321 GGAAGTATGC CGCATCTACG ACGGACCACT CAGATCGGTC GTTACGAGGG TTTGACTTTG  
10381 ACTGGACCAC CGACTCGTCT TCCACTGCCA GCGATACCAT GTCGCTACCC AGTTTGCACT  
10441 CGTGTGACAT CGACTCGATC TACGAGCCAA TGGCTCCCAT AGTAGTGACG GCTGACGTAC  
10501 ACCTGAACC CGCAGGCATC GCGGACCTGG CCGCAGATGT GCACCCTGAA CCCGCAGACC  
10561 ATGTGGACCT GGAGAACCCG ATTCCTCCAC CGCGCCCGAA GAGAGCTGCA TACCTTGCTT  
10621 CCCGCGCGGC GGAGCGACCG GTGCCGCGCG CGAGAAAGCC GACGCTGCC CCAAGGACTG  
10681 CGTTTAGGAA CAAGCTGCCT TTGACGTTTCG GCGACTTTGA CGAGCACGAG GTCGATGCGT  
10741 TGACCTCCGG GATTACTTTC GAGAGCTTCG ACGACGTCCT GCGACTAGGC CGCGCGGGTG  
10801 CATATATTTT CTCCTCGGAC ACTGGCAGCG GACATTTACA ACAAAAATCC GTTAGGCAGC  
10861 ACAATCTCCA GTGCGCACAA CTGGATGCGG TCCAGGAGGA GAAAATGTAC CCGCCAAAAT  
10921 TGATACTGA GAGGGAGAAG CTGTTGCTGC TGAATATGCA GATGCACCCA TCGGAGGCTA  
10981 ATAAGAGTCG ATACCAGTCT CGCAAAGTGG AGAATCATGAA AGCCACGGTG GTGGACAGGC  
11041 TCACATCGGG GGCCAGATTG TACACGGGAG CCGACGTAGG CCGCATACCA ACATACCGCG  
11101 TTCGGTACCC CCGCCCCGTG TACTCCCCTA CCGTGATCGA AAGATTCTCA AGCCCCGATG  
11161 TGCAATCCGC AGCTTGCAAC GAATACTTAT CCAGAAATTA CCAACAGTG CCGCTCGTACC  
11221 AGATAACAGA TGAATACGAC GCATACTTGG ACATGGTTGA CGGGTCGGAT AGTTGCTTGG  
11281 ACAGAGCGAC ATTCTGCCCG GCGAAGCTCC GGTGCTACCC GAAACATCAT GCGTACCACC  
11341 AGCCGACTGT ACGCAGTGCC GTCCCGTCAC CCTTTCAGAA CACACTACAG AACGTGCTAG  
11401 CGGCTGCCAC CAAGAGAAAC TGCAACGTCA CGCAAATGCG AGAACTACCC ACCATGGACT  
11461 CGGCACTGTT CAACGTGGAG TGCTTCAAGC GCTATGCCTG CTCCGGAGAA TATTGGGAAG  
11521 AATATGCTAA ACAACCTATC CGGATAACCA CTGAGAACAT CACTACCTAT GTGACCAAAT  
11581 TGAAAGGCCC GAAAGCTGCT GCCTTGTTTCG CTAAGACCCA CAACTTGGTT CCGCTGCAGG  
11641 AGGTTCCCAT GGACAGATTC ACGGTCGACA TGAACGAGA TGTCAAAGTC ACTCCAGGGA  
11701 CGAAACACAC AGAGGAAAGA CCCAAAGTCC AGGTAATTCA AGCAGCGGAG CCATTGGCGA  
11761 CCGCTTACCT GTGCGGCATC CACAGGGAAT TAGTAAGGAG ACTAAATGCT GTGTTACGCC  
11821 CTAACGTGCA CACATTGTTT GATATGTCGG CCGAAGACTT TGACGCGATC ATCGCCTCTC  
11881 ACTTCCACCC AGGAGACCCG GTTCTAGAGA CCGACATTGC ATCATTGAC AAAAGCCAGG  
11941 ACGACTCCTT GGCTCTTACA GGTTTAATGA TCCTCGAAGA TCTAGGGGTG GATCAGTACC  
12001 TGCTGGACTT GATCGAGGCA GCCTTTGGGG AAATATCCAG CTGTACCTA CCAACTGGCA  
12061 CGCGCTTCAA GTTCGGAGCT ATGATGAAAT CGGGCATGTT TCTGACTTTG TTTATTAACA  
12121 CTGTTTTGAA CATCACCATA GCAAGCAGGG TACTGGAGCA GAGACTCACT GACTCCGCTT  
12181 GTGCGGCCTT CATCGGCGAC GACAACATCG TTCACGGAGT GATCTCCGAC AAGCTGATGG  
12241 CGGAGAGGTG CGCGTCGTGG GTCAACATGG AGGTGAAGAT CATTGACGCT GTCATGGGCG  
12301 AAAAACCCCC ATATTTTTGT GGGGGATTCA TAGTTTTTGA CAGCGTCACA CAGACCGCCT  
12361 GCCGTGTTTC AGACCCACTT AAGCGCTGT TCAAGTTGGG TAAGCCGCTA ACAGCTGAAG  
12421 ACAAGCAGGA CGAAGACAGG CGACGAGCAC TGAGTGACGA GGT

FIGURE 29E





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## pDEST10 6708 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
23..152		Ppolh
461..337		attR1
711..1370		CmR
1490..1574		inactivated ccdA
1712..2017		ccdB
2058..2182		attR2
3394..4369		ampR
4510..5164		ori
5658..62		genR

1	CCCCGGATGA	AGTGGTTTCGC	ATCCTCGGTT	TTCTGGAAGG	CGAGCATCGT	TGTTTCGCCC
61	AGGACTCTAG	CTATAGTTCT	AGTGGTTGGC	TACGTATACT	CCGGAATATT	AATAGATCAT
121	GGAGATAATT	AAAATGATAA	CCATCTCGCA	AATAAATAAG	TATTTTACTG	TTTTCGTAAC
181	AGTTTGTAA	TAAAAAAACC	TATAAATATT	CCGGATTATT	CATACCGTCC	CACCATCGGG
241	CGCGGATCTC	GGTCCGAAAC	CATGTCGTAC	TACCATCACC	ATCACCATCA	CGATTACGAT
301	ATCCCAACGA	CCGAAAACCT	GTATTTTCAG	GGCATCACAA	GTTTGTACAA	AAAAGCTGAA
361	CGAGAAACGT	AAAATGATAT	AAATATCAAT	ATATTAAATT	AGATTTTGCA	TAAAAAACAG
421	ACTACATAAT	ACTGTAAAAC	ACAACATATC	CAGTCACTAT	GGCGGCCGCT	AAGTTGGCAG
481	CATCACCCGA	CGCACTTTGC	GCCGAATAAA	TACCTGTGAC	GGAAGATCAC	TTCGCAGAAT
541	AAATAAATCC	TGGTGTCCCT	GTTGATACCG	GGAAGCCCTG	GGCCAACCTT	TGGCGAAAAT
601	GAGACGTTGA	TCGGCACGTA	AGAGGTTCCA	ACTTTCACCA	TAATGAAATA	AGATCACTAC
661	CGGCGGTATT	TTTTGAGTTA	TCGAGATTTT	CAGGAGCTAA	GGAAGCTAAA	ATGGAGAAAA
721	AAATCACTGG	ATATACCACC	GTTGATATAT	CCCAATGGCA	TCGTAAAGAA	CATTTTGAGG
781	CATTTCAGTC	AGTTGCTCAA	TGTACCTATA	ACCAGACCGT	TCAGCTGGAT	ATTACGGCCT
841	TTTAAAGAC	CGTAAAGAAA	AATAAGCACA	AGTTTATATC	GGCCTTTATT	CACATTCTTG
901	CCCGCTGAT	GAATGCTCAT	CCGGAATTCC	GTATGGCAAT	GAAAGACGGT	GAGCTGGTGA
961	TATGGGATAG	TGTTACCCTT	TGTTACACCG	TTTTCCATGA	GCAAACCTGAA	ACGTTTTTCAT
1021	CGCTCTGGAG	TGAATACCAC	GACGATTTC	GGCAGTTTCT	ACACATATAT	TCGCAAGATG
1081	TGGCGTGTTA	CGGTGAAAAC	CTGGCCATT	TCCCTAAAGG	GTTTATTGAG	AATATGTTTT
1141	TCGTCTCAGC	CAATCCCTGG	GTGAGTTTCA	CCAGTTTTGA	TTTAAACGTG	GCCAATATGG
1201	ACAACCTTCT	CGCCCCGTT	TTCACCATGG	GCAAATATTA	TACGCAAGGC	GACAAGGTGC
1261	TGATGCCGCT	GGCGATTGAG	GTTTCATCAT	CCGTCTGTGA	TGGCTTCCAT	GTCGGCAGAA
1321	TGCTTAATGA	ATTACAACAG	TACTGCCATG	AGTGGCAGGG	CGGGGCGTAA	ACGCGTGGAT
1381	CCGGCTTACT	AAAAGCCAGA	TAACAGTATG	CGTATTTGCG	CGCTGATTTT	TGCGGTATAA
1441	GAATATATAC	TGATATGTAT	ACCCGAAGTA	TGTCAAAAAG	AGGTGTGCTA	TGAAGCAGCG
1501	TATTACAGTG	ACAGTTGACA	CGACAGCTA	TCAGTTGCTC	AAGGCATATA	TGATGTCAAT
1561	ATCTCCGGTC	TGGTAAGCAC	AACCATGCAG	AATGAAGCCC	GTCGTCTGCG	TGCCGAACGC
1621	TGGAAAGCGG	AAAATCAGGA	AGGGATGGCT	GAGGTCGCCC	GTTTATTGTA	AATGAACGGC
1681	TCTTTTGCTG	ACGAGAACAG	GGACTGGTGA	AATGCAGTTT	AAGGTTTACA	CCTATAAAAG
1741	AGAGAGCCGT	TATCGTCTGT	TTGTGGATGT	ACAGAGTGAT	ATTATTGACA	CGCCCGGGCG
1801	ACGGATGGTG	ATCCCCCTGG	CCAGTGCACG	TCTGCTGTCA	GATAAAGTCT	CCCGTGAACCT
1861	TTACCCGGTG	GTGCATATCG	GGGATGAAAG	CTGGCGCATG	ATGACCACCG	ATATGGCCAG
1921	TGTGCCGGTC	TCCGTTATCG	GGGAAGAAGT	GGCTGATCTC	AGCCACCGCG	AAAATGACAT
1981	CAAAAACGCC	ATTAACCTGA	TGTTCTGGGG	AATATAAATG	TCAGGCTCCC	TTATACACAG
2041	CCAGTCTGCA	GGTCGACCAT	AGTGACTGGA	TATGTTGTGT	TTTACAGTAT	TATGTAGTCT
2101	GTTTTTTATG	CAAAATCTAA	TTTAATATAT	TGATATTTAT	ATCATTTTAC	GTTTCTCGTT
2161	CAGCTTTCTT	GTACAAAGTG	GTGATGCCAT	GGATCCGGAA	TTCAAAGGCC	TACGTCGACG
2221	AGCTCAACTA	GTGCGGCCGC	TTTCGAATCT	AGAGCCTGCA	GTCCTGAGGC	ATGCGGTACC
2281	AAGCTTGTCT	AGAAGTACTA	GAGGATCATA	ATCAGCCATA	CCACATTTGT	AGAGGTTTTA
2341	CTTGCTTTAA	AAAACCTCCC	ACACCTCCCC	CTGAACCTGA	AACATAAAAT	GAATGCAATT
2401	GTTGTTGTTA	ACTTGTTTAT	TGCAGTTTAT	AATGGTTACA	AATAAAGCAA	TAGCATCACA
2461	AATTTACAA	ATAAAGCATT	TTTTCACTG	CATTCTAGTT	GTGGTTTGTC	CAAACTCATC
2521	AATGTATCTT	ATCATGTCTG	GATCTGATCA	CTGCTTGAGC	CTAGGAGATC	CGAACCAGAT
2581	AAGTGAAATC	TAGTTCCAAA	CTATTTTGTC	ATTTTAAATT	TTCGTATTAG	CTTACGACGC-

FIGURE 30B

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2641 TACACCCAGT TCCCATCTAT TTTGTCACTC TTCCCTAAAT AATCCTTAAA AACTCCATTT  
2701 CCACCCCTCC CAGTTCCCAA CTATTTTGTG CGCCACAGC GGGGCATTTT TCTTCCTGTT  
2761 ATGTTTTTAA TCAAACATCC TGCCAACTCC ATGTGACAAA CCGTCATCTT CGGCTACTTT  
2821 TTCTCTGTCA CAGAATGAAA ATTTTCTGT CATCTCTCG TTATTAATGT TTGTAATTGA  
2881 CTGAATATCA ACGCTTATTT GCAGCCTGAA TGGCGAATGG GACGCGCCCT GTAGCGGCGC  
2941 ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG CCAGCGCCCT  
3001 AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC ACGTTCGCGG GCTTTCCCGG  
3061 TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT AGTGCTTTAC GGCACCTCGA  
3121 CCCCAAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT GATAGACGGT  
3181 TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT TCCAAACTGG  
3241 AACAACTCTC AACCCTATCT CGGTCTATTC TTTTGATTGA TAAGGGATTT TGCCGATTTT  
3301 GGCCTATTGG TTAAAAATG AGCTGATTTA AAAAAAATTT AACCGGAATT TTAACAAAAT  
3361 ATTAACGTTT ACAATTTTCA GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG  
3421 TTTATTTTTT TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT  
3481 GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT  
3541 TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT  
3601 AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC ATCGAACTGG ATCTCAACAG  
3661 CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA GCACTTTTAA  
3721 AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTGCG  
3781 CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT  
3841 TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC  
3901 TGCGGCCAAC TACTTCTGTA CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA  
3961 CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT  
4021 ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT TGCGCAAACCT  
4081 ATTAACCTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC  
4141 GGATAAAGTT GCAGGACCAC TTCTGCCTC GGCCTTCCG GCTGGCTGGT TTATTGCTGA  
4201 TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG  
4261 TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG  
4321 AAATAGACAG ATCGCTGAGA TAGGTGCTC ACTGATTAAG CATTGGTAAC TGTCAGACCA  
4381 AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA  
4441 GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTTCGTTCCA  
4501 CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG  
4561 CGTAATCTGC TGCTTGCAA CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA  
4621 TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAAA  
4681 TACTGTCTTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC  
4741 TACATACCTC GCTCTGCTAA TCCTGTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCTG  
4801 TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT CGGGCTGAAC  
4861 GGGGGGTTTC TGCACACAGC CCAGCTTGA GCGAACGACC TACACCGAAC TGAGATACCT  
4921 ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC  
4981 GGTAAAGCGG AGGGTCCGAA CAGGAGAGCG CACGAGGGG CTTCCAGGGG GAAACGCTCG  
5041 GTATCTTTAT AGTCCTGTG GGTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG  
5101 CTCGTCAGGG GGGCGGAGCC TATGGAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCTT  
5161 GGCTTTTTCG TGGCCTTTTG CTCACATGTT CTTTCCTGCG TTATCCCTG ATTCTGTGGA  
5221 TAACCGTATT ACCGCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA CGACCGAGCG  
5281 CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCTGATG CGGTATTTTC TCCTTACGCA  
5341 TCTGTGCGGT ATTTACACAC GCAGACCAGC GCGTAACCT GGCAAAATCG GTTACGGTTG  
5401 AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGGCGGA CAATAAAGTC TTAAGTGAA  
5461 CAAAATAGAT CTAAGTATG ACAATAAAGT CTTAAACTAG ACAGAATAGT TGTAACTGA  
5521 AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGACTTT TGTTATGGCT AAAGCAAACCT  
5581 CTTCATTTTT TGAAGTGCAA ATTGCCCCTG GTATTAAAGA GGGGCGTGGC CAAGGGCATG  
5641 GTAAAGACTA TATTGCGCGC GTTGTGACAA TTTACCGAAC AACTCCGCGG CCGGGAAGCC  
5701 GATCTCGGCT TGAACGAATT GTTAGGTGGC GGTACTTGGG TCGATATCAA AGTGATCAC  
5761 TTCTTCCCGT ATGCCCACT TTGTATAGAG AGCCACTGCG GGATCGTCAC CGTAATCTGC  
5821 TTGCACGTAG ATCACAAG CACCAAGCGC GTTGGCCTCA TGCTTGAGGA GATTGATGAG  
5881 CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCGGAGACT GCGAGATCAT AGATATAGAT  
5941 CTCACTACGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC GCGAGAGCGC CAACAACCGC  
6001 TTCTTGGTTC AAGGCAGCAA GCGCGATGAA TGTCTTACTA CGGAGCAAGT TCCCGAGGTA  
6061 ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT CCGAACTCAC GACCGAAAAG-

FIGURE 30C

6121 ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGGCCG AGCCTACATG TCGGAATGAT  
6181 GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG CCCTGCTGCG TAACATCGTT  
6241 GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA TCGACCCACG GCGTAACGCG  
6301 CTTGCTGCTT GGATGCCCCG GGCATAGACT GTACAAAAA ACAGTCATAA CAAGCCATGA  
6361 AAACCGCCAC TGCGCCGTTA CCACCGCTGC GTTCGGTCAA GGTTCCTGGAC CAGTTGCGTG  
6421 AGCGCATACG CTAATTGCAT TACAGTTTAC GAACCGAACA GGCTTATGTC AACTGGGTTC  
6481 GTGCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC CTTGGGCAGC AGCGAAGTCG  
6541 AGGCATTTCT GTCCTGGCTG GCGAACGAGC GCAAGGTTTC GGTCTCCACG CATCGTCAGG  
6601 CATTGGCGGC CTTGCTGTTC TTCTACGGCA AGGTGCTGTG CACGGATCTG CCCTGGCTTC  
6661 AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGCCGGT GGTGCTGA

FIGURE 30D

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Figure 31A:

pDEST11

**Tet-regulated eukaryotic expression**

358 tag tga acc ggc aga tgc cct gga gac gcc atc cac gct gtt tgc acc tcc  
 atc act tgg cag tct agc gga cct ctg cgg tag gtg cga caa aac tgg agg

409 ata gaa gac acc ggg acc gat cca gcc tcc gcg gcc ccg aat tgc agc tgc  
 tat ctt ctg tgg ccc tgg cta ggt cgg agg cgc cgg ggc tta agc tgc agc

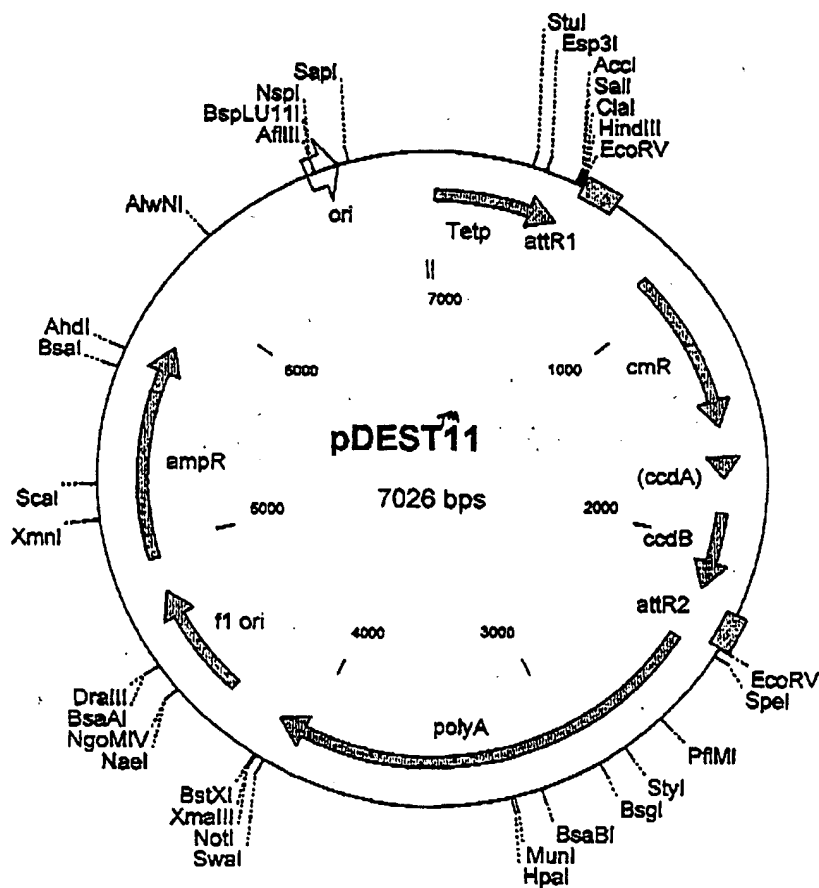
460 gta ccc ggg gat cct cta gag tgc agg tgc acg gta tgc ata agc tgc agc  
 cat ggg ccc cta gga gat ctc agc tcc agc tgc cat agc tat tgc agc tat

511 tca aca agt tgc tgc tgc tgc tgc tgc tgc tgc tgc tgc tgc tgc tgc tgc  
 agt tgc tca aac atg ttt tgc tgc tgc tgc tgc tgc tgc tgc tgc tgc tgc

*mRNA from CMV promoter (controlled by tetracycline)*

*SalI* *ClaI* *Hind3* *EcoRV*

*Int* *attR1*



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## pDEST11 7026 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
4..479		Tetp ((Tet operator) 7 and min hCMV promoter)
638..514		attR1
888..1547		CmR
1667..1751		inactivated ccdA
1889..2194		ccdB
2235..2359		attR2
2402..4132		polyA
4347..4803		f1 ori
4940..5797		ampR
1	CGAGTTTACC ACTCCCTATC AGTGATAGAG AAAAGTGAAA GTCGAGTTTA CCACTCCCTA	
61	TCAGTGATAG AGAAAAGTGA AAGTCGAGTT TACCACTCCC TATCAGTGAT AGAGAAAAGT	
121	GAAAGTCGAG TTTACCACTC CCTATCAGTG ATAGAGAAAA GTGAAAGTCG AGTTTACCAC	
181	TCCCTATCAG TGATAGAGAA AAGTGAAAGT CGAGTTTACC ACTCCCTATC AGTGATAGAG	
241	AAAAGTGAAA GTCGAGTTTA CCACTCCCTA TCAGTGATAG AGAAAAGTGA AAGTCGAGCT	
301	CGGTACCCGG GTCGAGTAGG CGGTGACGGT GGGAGGCCTA TATAAGCAGA GTCGTTTTAG	
361	TGAACCGTCA GATCGCCTGG AGACGCCATC CACGCTGTTT TGACCTCCAT AGAAGACACC	
421	GGGACCGATC CAGCCTCCGC GGCCCCGAAT TCGAGCTCGG TACCCGGGGA TCCTCTAGAG	
481	TCGAGGTCGA CGGTATCGAT AAGCTTGATA TCAACAAGTT TGTACAAAA AGCTGAACGA	
541	GAAACGTAAA ATGATATAAA TATCAATATA TTAAATTAGA TTTTGATAA AAAACAGACT	
601	ACATAATACT GTAAACACAC ACATATCCAG TCACATGGC GGCCGCTAAG TTGGCAGCAT	
661	CACCCGACGC ACTTTGCGCC GAATAAATAC CTGTGACGGA AGATCACTTC GCAGAATAAA	
721	TAAATCCTGG TGTCCTGTT GATACCGGGA AGCCCTGGGC CAACTTTTGG CGAAAATGAG	
781	ACGTTGATCG GCACGTAAGA GGTTCCAACT TTCACCATAA TGAAATAAGA TCACTACCGG	
841	GCGTATTTTT TGAGTTATCG AGATTTTCAG GAGCTAAGGA AGCTAAAATG GAGAAAAAAA	
901	TCAGTGATA TACCACCGTT GATATATCCC AATGGCATCG TAAAGAACAT TTTGAGGCAT	
961	TTCACTCAGT TGCTCAATGT ACCTATAACC AGACCGTTCA GCTGGATATT ACGGCCTTTT	
1021	TAAAGACCGT AAAGAAAAAT AAGCACAAGT TTTATCCGGC CTTTATTCAC ATTCTGCC	
1081	GCCTGATGAA TGCTATCCG GAATTCGGTA TGGCAATGAA AGACGGTGAG CTGGTGATAT	
1141	GGGATAGTGT TCACCCTTGT TACACCGTTT TCCATGAGCA AACTGAAACG TTTTCATCGC	
1201	TCTGGAGTGA ATACCACGAC GATTTCCGGC AGTTTCTACA CATATATTCC CAAGATGTGG	
1261	CGTGTACGG TGAAACCTG GCCTATTTCC CTAAAGGGTT TATTGAGAAT ATGTTTTTCG	
1321	TCTCAGCCAA TCCCTGGGTG AGTTTCACCA GTTTTGATTT AAACGTGGCC AATATGGACA	
1381	ACTTCTTCGC CCCCCTTTTC ACCATGGGCA AATATTATAC GCAAGGCGAC AAGGTGCTGA	
1441	TGCCGTGGC GATTCAGTT CATCATGCCG TCTGTGATGG CTTCCATGTC GGCAGAATGC	
1501	TTAATGAATT ACAACAGTAC TGCGATGAGT GGCAGGGCGG GGCGTAAAGA TCTGGATCCG	
1561	GCTTACTAAA AGCCAGATAA CAGTATGCGT ATTTGCGCGC TGATTTTTGC GGTATAAGAA	
1621	TATATACTGA TATGTATACC CGAAGTATGT CAAAAAGAGG TGTGCTATGA AGCAGCGTAT	
1681	TACAGTGACA GTTGACAGCG ACAGCTATCA GTTGCTCAAG GCATATATGA TGTCATATC	
1741	TCCGGTCTGG TAAGCACAAC CATGCAGAAT GAAGCCCGTC GTCTGCGTGC CGAACGCTGG	
1801	AAAGCGGAAA ATCAGGAAGG GATGGCTGAG GTCGCCCCGT TTATTGAAAT GAACGGCTCT	
1861	TTTGCTGACG AGAACAGGGA CTGGTGAAAT GCAGTTTAAG GTTTACACCT ATAAAAGAGA	
1921	GAGCCGTTAT CGTCTGTTTG TGGATGTACA GAGTGATATT ATTGACACGC CCGGGCGACG	
1981	GATGGTGATC CCCCTGGCCA GTGCACGTCT GCTGTGAGAT AAAGTCTCCC GTGAACCTTA	
2041	CCCGGTGGTG CATATCGGGG ATGAAAGCTG GCGCATGATG ACCACCGATA TGGCCAGTGT	
2101	GCCGGTCTCC GTTATCGGGG AAGAAGTGGC TGATCTCAGC CACCGCGAAA ATGACATCAA	
2161	AAACGCCATT AACCTGATGT TCTGGGGAAT ATAAATGTCA GGCTCCCTTA TACACAGCCA	
2221	GTCTGCAGGT CGACCATAGT GACTGGATAT GTTGTGTTT ACAGTATTAT GTAGTCTGTT	
2281	TTTTATGCAA AATCTAATTT AATATATTGA TATTTATATC ATTTTACGTT TCTCGTTCTA	
2341	CTTTCTTGTA CAAAGTGGTT GATATCGAAT TCCTGCAGCC CGGGGGATCC ACTAGTTCTA	
2401	GATCACTGCG ATGAGTGGCA GGGCGGGCGG TAATTTTTTT AAGGCAGTTA TTGGTGCCCT	
2461	TAAACGCCCTG GTGCTACGCC TGAATAAGTG ATAATAAGCG GATGAATGGC AGAAATTCGC	
2521	CGGATCTTTG TGAAGGAACC TTAATTCTGT GGTGTGACAT AATTGGACAA ACTACCTACA-	

FIGURE 31B

2581 GAGATTTAAA GCTCTAAGGT AAATATAAAA TTTTAAAGTG TATAATGTGT TAAACTACTG  
2641 ATTCTAATTG TTTGTGTATT TTAGATTCCA ACCTATGGAA CTGATGAATG GGAGCAGTGG  
2701 TGGAAATGCCT TTAATGAGGA AAACCTGTTT TGCTCAGAAG AAATGCCATC TAGTGATGAT  
2761 GAGGCTACTG CTGACTCTCA ACATTCTACT CCTCCAAAAA AGAAGAGAAA GGTAGAAGAC  
2821 CCCAAGGACT TTCCTTCAGA ATTGCTAAGT TTTTGTAGTC ATGCTGTGTT TAGTAATAGA  
2881 ACTCTTGCTT GCTTTGCTAT TTACACCACA AAGGAAAAAG CTGCACTGCT ATACAAGAAA  
2941 ATTATGAAAA AATATTCTGT AACCTTTATA AGTAGGCATA ACAGTTATAA TCATAACATA  
3001 CTGTTTTTTC TTAATCCACA CAGGCATAGA GTGCTGTGCTA TTAATAACTA TGCTCAAAAA  
3061 TTGTGTACCT TTAGCTTTTT AATTTGTAAA GGGGTTAATA AGGAATATTT GATGTATAGT  
3121 GCCTTGACTA GAGATCATAA TCAGCCATAC CACATTTGTA GAGGTTTTAC TTGCTTTAAA  
3181 AAACCTCCCA CACCTCCCCC TGAACCTGAA ACATAAAATG AATGCAATTG TTGTTGTTAA  
3241 CTTGTTTTATT GCAGCTTATA ATGGTTACAA ATAAAGCAAT AGCATCACAA ATTTCCACAA  
3301 TAAAGCATTT TTTTCACTGC ATTCTAGTTG TGGTTTGTCC AAACCTCATCA ATGTATCTTA  
3361 TCATGTCTGG ATCCCCAGGA AGCTCCTCTG TGTCTCATA AACCTTAACC TCCTCTACTT  
3421 GAGAGGACAT TCCAATCATA GGCTGCCCAT CCACCCTCTG TGTCTCTCTG TTAATTAGGT  
3481 CACTTAACAA AAAGGAAATT GGGTAGGGGT TTTTCACAGA CCGCTTTCTA AGGGTAATTT  
3541 TAAAATATCT GGGAAAGTCCC TTCACTGCT GTGTTCCAGA AGTGTGGTA AACAGCCAC  
3601 AAATGTCAAC AGCAGAAACA TACAAGCTGT CAGCTTTGCA CAAGGGCCCA ACACCCTGCT  
3661 CATCAAGAAG CACTGTGGTT GCTGTGTTAG TAATGTGCAA AACAGGAGGC ACATTTTCCC  
3721 CACCTGTGTA GGTTCACAAA TATCTAGTGT TTTTATTTT ACTTGATCA GGAACCCAGC  
3781 ACTCCACTGG ATAAGCATTG TCCTTATCCA AAACAGCCTT GTGGTCAGTG TTCATCTGCT  
3841 GACTGTCAAC TGTAGCATTT TTTGGGGTTA CAGTTTGAGC AGGATATTTG GTCCTGTAGT  
3901 TTGCTAACAC ACCCTGCAGC TCCAAAGGTT CCCACCAAC AGCAAAAAAA TGAAAATTTG  
3961 ACCCTTGAAT GGGTTTCCCA GCACCATTTT CATGAGTTTT TTGTGTCCTT GAATGCAAGT  
4021 TTAACATAGC AGTTACCCCA ATAACCTCAG TTTTAAACAGT AACAGCTTCC CACATCAAAA  
4081 TATTTCCACA GGTAAAGTCC TCATTTAAAT TAGGCAAAGG AATTGCTCTA GAGCGGCCGC  
4141 CACCGCGGTG GAGCTCCAAT TCGCCCTATA GTGAGTCGTA TTACGCGCGC TCACTGGCCG  
4201 TCGTTTTACA ACGTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG  
4261 CACATCCCCC TTTCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCC  
4321 AACAGTTGCG CAGCCTGAAT GGCGAATGGG ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG  
4381 CGGTGTGGT GGTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA CGCGCCGCTC  
4441 TTTTCGCTTT CTTCCCTTCC TTCTCGCCA CGTTCGCCGG CTTTCCCGT CAAGCTCTAA  
4501 ATCGGGGGCT CCCTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC  
4561 TTGATTAGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTGCGCCTT  
4621 TGACGTTGGA GTCCACGTTT TTAATAGTG GACTCTTGTT CCAAAGTGA ACAACACTCA  
4681 ACCCTATCTC GGTCTATTCT TTGATTTTAT AAGGGATTTT GCCGATTTG GCTATTGGT  
4741 TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGCTTA  
4801 CAATTTAGGT GGCACCTTTC GGGGAAATGT GCGCGGAACC CCTATTTGTT TATTTTCTA  
4861 AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC TTCAATAATA  
4921 TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CCTTTTTTGC  
4981 GGCATTTTGC CTTCTGTTT TTGCTCACCC AGAAACGCTG GTGAAAGTAA AAGATGCTGA  
5041 AGATCAGTTG GGTGCACGAG TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT  
5101 TGAGAGTTTT CGCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG  
5161 TGGCGCGGTA TTATCCCGTA TTGACGCCGG GCAAGAGCAA CTCGGTCGCC GCATACACTA  
5221 TTCTCAGAAT GACTTGGTTG AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT  
5281 GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACTT  
5341 ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCGCT TTTTGCACA ACATGGGGGA  
5401 TCATGTAACG CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA  
5461 GCGTGACACC ACGATGCCTG TAGCAATGGC AACCAACGTT CGCAACTAT TAACTGGCGA  
5521 ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC  
5581 AGGACCACTT CTGCGCTCGG CCCTTCCGGC TGGCTGTTT ATTGCTGATA AATCTGGAGC  
5641 CGGTGAGCGT GGGTCTCGCG GTATCATTCG AGCACTGGGG CCAGATGGTA AGCCCTCCCCG  
5701 TATCGTAGTT ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT  
5761 CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA  
5821 TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT  
5881 TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCACT GAGCGTCAGA  
5941 CCCCCTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG  
6001 CTTGCAACAA AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC-

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6061 AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT  
6121 AGTGTAGCEG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACCTCGC  
6181 TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT  
6241 GGA CTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTG GGTGAACGG GGGGTTCTG  
6301 CACACAGCCC AGCTTGGAGC GAACGACCTA CACCGAAGT AGATACCTAC AGCGTGAGCT  
6361 ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG  
6421 GGTGCGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG  
6481 TCCTGTCGGG TTTCGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG  
6541 GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CCTTTTGCTG  
6601 GCCTTTTGCT CACATGTTCT TTCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC  
6661 CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT  
6721 GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT CTCCCCGCGC GTTGGCCGAT  
6781 TCATTAATGC AGCTGGCAGC ACAGGTTTCC CGACTGGAAA GCGGGCAGTG AGCGCAACGC  
6841 AATTAATGTG AGTTAGCTCA CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC  
6901 TCGTATGTTG TGTGGAATTG TGAGCGGATA ACAATTTTAC ACAGGAAACA GCTATGACCA  
6961 TGATTACGCC AAGCGCGCAA TTAACCCCTCA CTAAAGGGAA CAAAAGCTGG GTACCGGGCC  
7021 CCCCCT

FIGURE 31D

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**Figure 32A: pDEST12.2 CMV Promoter for Eukaryotic Expression, SV40 Promoter/ori for G418 Resistance**

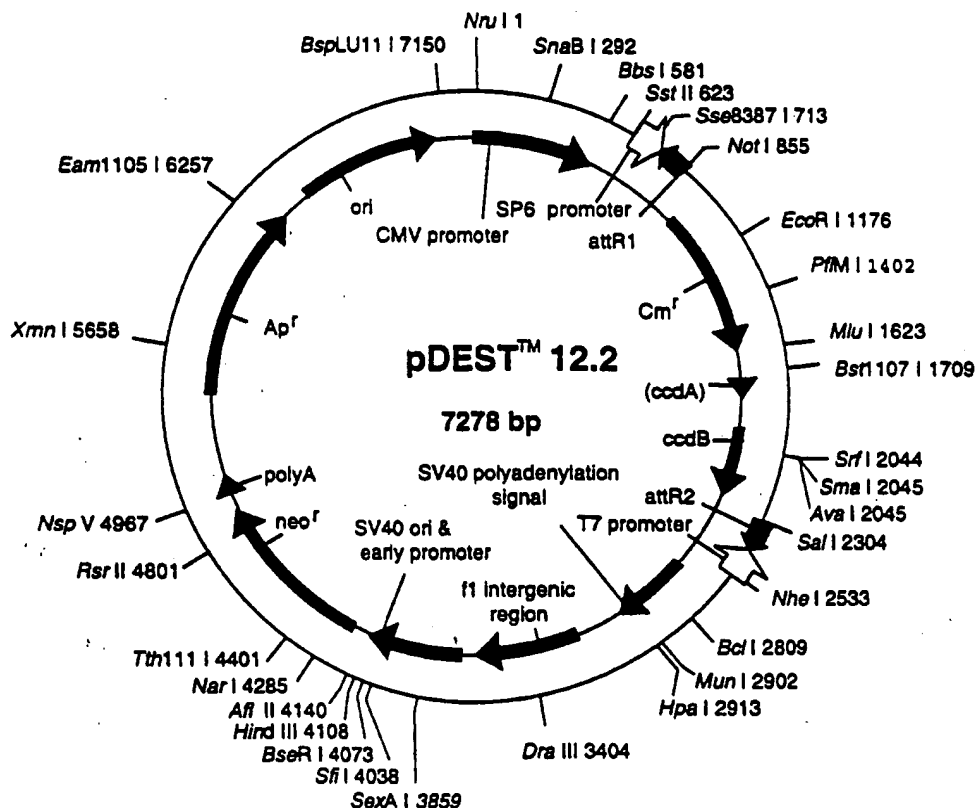
307 *→ mRNA from CMV promoter*  
 acc gtc aga tcg cct gga gac gcc atc cac gct gtt ttg acc tcc ata gaa  
 tgg cag tct agc gga cct ctg cgg tag gtg cga caa aac tgg agg tat ctt

358 gac acc ggg acc gat cca gcc tcc gga ctc tag cct agg ccg cgg agc gga  
 ctg tgg ccc tgg cta ggt cgg agg cct gag atc gga tcc ggc gcc tcg cct

409 taa caa ttt cac aca gga aac agc tat gac cat tag gcc ttt gca aaa agc  
 att gtt aaa gtg tgt cct ttg tcg ata ctg gta atc cgg aaa cgt ttt tcg

460 tat tta ggt gac act ata gaa ggt acg cct gca ggt acc ggt ccg gaa ttc  
 ata aat cca ctg tga tat ctt cca tgc gga cgt cca tgg cca ggc ctt aag  
*Int attR1*

511 cca tca aca agt ttg taa ada gaa gct gaa cga gaa acg taa aat gat ata  
 ggt agt tgt tca aac atg ttg ttt cga ctt gct ctt tgc att tta cta tat





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## pDEST12.2 7278 bp (rotated to position 3900)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
86..136		ori
220..742		CMV promoter
1059..935		attR1
1168..1827		CmR
1947..2031		inactivated ccdA
2169..2474		ccdB
2515..2639		attR2
2824..3186		small t & polyA
3310..3378		lac
4363..5157		neo
5680..6540		ampR

1	GGGGGGCGGA	GCCTATGGAA	AAACGCCAGC	AACGCGGCCT	TTTTACGGTT	CCTGGCCTTT
61	TGCTGGCCTT	TTGCTCACAT	GTTCTTTCCT	GCGTTATCCC	CTGATTCTGT	GGATAACCGT
121	ATTACCGCCT	TTGAGTGAGC	TGATACCGCT	CGCCGCAGCC	GAACGACCGA	GCGCAGCGAG
181	TCAGTGAGCG	AGGAAGCGGA	AGAGCTCGCG	AATGCATGTC	GTTACATAAC	TTACGGTAAA
241	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTTG	ACGTCAATAA	TGACGTATGT
301	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGAGT	ATTTACGGTA
361	AACTGCCCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	CTATTGACGT
421	CAATGACGGT	AAATGGCCCCG	CCTGGCATT	TGCCCAGTAC	ATGACCTTAT	GGGACTTTCC
481	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	GGTTTTGGCA
541	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	TCCACCCCAT
601	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	AATGTCGTAA
661	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	TCTATATAAG
721	CAGAGCTCGT	TTAGTGAACC	GTCAGATCGC	CTGGAGACGC	CATCCACGCT	GTTTTGACCT
781	CCATAGAACT	CACCGGGACC	GATCCAGCCT	CCGGACTCTA	GCCTAGGCCG	CGGGACGGAT
841	AACAATTTCA	CACAGGAAAC	AGCTATGACC	ATTAGGCCTT	TGCAAAAAGC	TATTTAGGTG
901	ACACTATAGA	AGGTACGCCT	GCAGGTACCG	GATCACAAGT	TTGTACAAAA	AAGCTGAACG
961	AGAAACGTAA	AATGATATAA	ATATCAATAT	ATTAAATTAG	ATTTTGCCATA	AAAAACAGAC
1021	TACATAATAC	TGTAAAACAC	AACATATCCA	GTCACATATG	CGGCCGCATT	AGGCACCCCA
1081	GGCTTTACAC	TTTATGCTTC	CGGCTCGTAT	AATGTGTGGA	TTTTGAGTTA	GGATCCGTCG
1141	AGATTTTCAG	GAGCTAAGGA	AGCTAAAATG	GAGAAAAAAA	TCACTGGATA	TACCACCGTT
1201	GATATATCCC	AATGGCATCG	TAAAGAACAT	TTTGAGGCAT	TTCAAGTCAGT	TGCTCAATGT
1261	ACCTATAACC	AGACCGTTCA	GCTGGATATT	ACGGCCTTTT	TAAAGACCGT	AAAGAAAAAT
1321	AAGCACAAGT	TTTATCCGGC	CTTTATTAC	ATTCTTGCCC	GCCTGATGAA	TGCTCATCCG
1381	GAATTCGGTA	TGGCAATGAA	AGACGGTGAG	CTGGTGATAT	GGGATAGTGT	TCACCCCTGT
1441	TACACCGTTT	TCCATGAGCA	AACTGAAACG	TTTTTCATCGC	TCTGGAGTGA	ATACCACGAC
1501	GATTTCCGGC	AGTTTCTACA	CATATATTCG	CAAGATGTGG	CGTGTTACGG	TGAAAACCTG
1561	GCCTATTTCC	CTAAAGGGTT	TATTGAGAA	ATGTTTTTTCG	TCTCAGCCAA	TCCCTGGGTG
1621	AGTTTCACCA	GTTTTGATTT	AAACGTGGCC	AATATGGACA	ACTTCTTCGC	CCCCGTTTTT
1681	ACCATGGGCA	AATATTATAC	GCAAGGCGAC	AAGGTGCTGA	TGCCGCTGGC	GATTCAGGTT
1741	CATCATGCCG	TCTGTGATGG	CTTCCATGTC	GGCAGAATGC	TTAATGAATT	ACAACAGTAC
1801	TGCGATGAGT	GGCAGGGCGG	GGCGTAAACG	CGTGATCCG	GCTTACTAAA	AGCCAGATAA
1861	CAGTATGCGT	ATTTGCGCGC	TGATTTTTGC	GGTATAAGAA	TATATACTGA	TATGTATACC
1921	CGAAGTATGT	CAAAAAGAGG	TGTGCTATGA	AGCAGCGTAT	TACAGTGACA	GTTGACAGCG
1981	ACAGCTATCA	GTTGCTCAAG	GCATATATGA	TGTCAATATC	TCCGGTCTGG	TAAGCACAAC
2041	CATGCAGAAT	GAAGCCCGTC	GTCTGCGTGC	CGAACGCTGG	AAAGCGGAAA	ATCAGGAAGG
2101	GATGGCTGAG	GTCGCGCGGT	TTATTGAAAT	GAACGGCTCT	TTTGCTGACG	AGAACAGGGA
2161	CTGGTGAAAT	GCAGTTTAAG	GTTTACACCT	ATAAAAGAGA	GAGCCGTTAT	CGTCTGTTTG
2221	TGGATGTACA	GAGTGATATT	ATTGACACGC	CCGGGCGACG	GATGGTGATC	CCCCTGGCCA
2281	GTGCACGTCT	GCTGTCTAGT	AAAGTCTCCC	GTGAACTTTA	CCCGGTGGTG	CATATCGGGG
2341	ATGAAAGCTG	GCGCATGATG	ACCACCGATA	TGGCCAGTGT	GCCGGTCTCC	GTTATCGGGG
2401	AAGAAGTGGC	TGATCTCAGC	CACCGCGAAA	ATGACATCAA	AAACGCCATT	AACCTGATGT-

FIGURE 32B

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2461 TCTGGGGAAT ATAAATGTCA GGCTCCCTTA TACACAGCCA GTCTGCAGGT CGACCATAGT  
2521 GACTGGATAT GTTGTGTTTT ACAGTATTAT GTAGTCTGTT TTTTATGCAA AATCTAATTT  
2581 AATATATTGA TATTTATATC ATTTTACGTT TCTCGTTCAG CTTTCTTGTA CAAAGTGGTG  
2641 ATCGCGTGCA TCGGACGTCA TAGCTCTCTC CCTATAGTGA GTCGTATTAT AAGCTAGGCA  
2701 CTGGCCGTCG TTTTACAACG TCGTGACTGG GAAAACGTCT AGCTTGGGAT CTTTGTGAAG  
2761 GAACCTTACT TCTGTGGTGT GACATAATTG GACAACTAC CTACAGAGAT TTAAAGCTCT  
2821 AAGGTAAATA TAAAATTTTT AAGTGATATA TGTGTTAAAC TAGCTGCATA TGCTTGCTGC  
2881 TTGAGAGTTT TGCTTACTGA GTATGATTTA TGAAAATATT ATACACAGGA GCTAGTGATT  
2941 CTAATTGTTT GTGTATTTTA GATTCACAGT CCCAAGGCTC ATTTTCAGGCC CCTCAGTCCT  
3001 CACAGTCTGT TCATGATCAT AATCAGCCAT ACCACATTTG TAGAGGTTTT ACTTGCTTTA  
3061 AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTTGTGTGT  
3121 AACTTGTTTT TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTACACA  
3181 AATAAAGCAT TTTTTTCACT GCATTCTAGT TGTGGTTTTG CCAAATCAT CAATGTATCT  
3241 TATCATGTCT GGATCGATCC TGCATTAAATG AATCGGCCAA CGCGCGGGGA GAGGCGGTTT  
3301 CGGTATTGGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCC AACAGTTGCG  
3361 CAGCCTGAAAT GCGCAATGGG ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT  
3421 GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT  
3481 CTTCCCTTCC TTTCTCGCCA CGTTCGCGCG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT  
3541 CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTAGGG  
3601 TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCCGCCCT TGACGTTGGA  
3661 GTCCACGTTT TTTAATAGTG GACTCTTGTT CCAAACCTGGA ACAACACTCA ACCCTATCTC  
3721 GGTCTATTCT TTTGATTTAT AAGGGATTTT GCCGATTTG GCCTATTGGT TAAAAAATGA  
3781 GCTGATTTAA CAAATATTTA ACGCGAATTT TAACAAATA TTAACGTTTA CAATTTCCGC  
3841 TGATGCGGTA TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA CGCGGATCTG  
3901 CGCAGCACCA TGGCCTGAAA TAACCTCTGA AAGAGGAACT TGGTTAGGTA CTTTCTGAGG  
3961 CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC  
4021 AGCAGGCAGA AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAGTC  
4081 CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAT  
4141 AGTCCCGCCC CTAACCTCCG CCACTCCCGC CCTAACCTCG CCCAGTTCCG CCCATTCTCC  
4201 GCCCATGCGC TGACTAATTT TTTTATTTA TGCAGAGGCC GAGGCCGCTT GCGCTTCTGA  
4261 GCCTATTCCG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTTGA  
4321 TTCTTCTGAC ACAACAGTCT CGAACCTAAG GCTAGAGCCA CCATGATTGA ACAAGATGGA  
4381 TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT TCGGCTATGA CTGGGCACAA  
4441 CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT CAGCGCAGGG GCGCCCGGTT  
4501 CTTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC TGCAGGACGA GGCAGCGCGG  
4561 CTATCGTGGC TGGCCACGAC GGGCGTTCCT TGCGCAGCTG TGCTCGACGT TGCTCACTGAA  
4621 GCGGGAAGGG ACTGGCTGCT ATTGGGCGAA GTGCCGGGGC AGGATCTCCT GTCATCTCAC  
4681 CTTGCTCCTG CCGAGAAAGT ATCCATCATG GCTGATGCAA TGCGGCGGCT GCATCGCTTT  
4741 GATCCGCGTA CCTGCCCATT CGACCACCAA GCGAAACATC GCATCGAGCG AGCACGTACT  
4801 CGGATGGAAG CCGGTCTTGT CGATCAGGAT GATCTGGACG AAGAGCATCA GGGGCTCGCG  
4861 CCAGCCGAAC TGTTCCGCCAG GCTCAAGGCG CGCATGCCCC ACGGCGAGGA TCTCGTCTGT  
4921 ACCCATGGCG ATGCCTGCTT GCCGAATATC ATGGTGGAAG ATGGCCGCTT TTCTGGATTC  
4981 ATCGACTGTG GCCGGCTGGG TGTGGCGGAC CGCTATCAGG ACATAGCGTT GGCTACCCGT  
5041 GATATTGCTG AAGAGCTTGG CCGCGAATGG GCTGACCGCT TCCTCGTGCT TTACGGTATC  
5101 GCCGCTCCCG ATTCGCAGCG CATCGCCTTC TATCGCCTTC TTGACGAGTT CTTCTGAGCG  
5161 GGA CTCTGGG GTTCGAAATG ACCGACCAAG CGACGCCCAA CCTGCCATCA CGATGGCCGC  
5221 AATAAAATAT CTTTATTTTC ATTACATCTG TGTGTTGGTT TTTTGTGTGA ATCGATAGCG  
5281 ATAAGGATCC GCGTATGGTG CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC  
5341 CAGCCCCGAC ACCCGCCAAC ACCCGCTGAC GCGCCCTGAC GGGCTTGTCT GCTCCCGGCA  
5401 TCCGCTTACA GACAAGCTGT GACCGTCTCC GGGAGCTGCA TGTGTAGAG GTTTTCACCG  
5461 TCATCACCAG AACGCGCGAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT  
5521 GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT TTCGGGGAAA TGTGCGCGGA  
5581 ACCCTATTTT GTTTATTTTT CTAAATATGT TCAAATATGT ATCCGCTCAT GAGACAATAA  
5641 CCCTGATAAA TGCTTCAATA ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT  
5701 GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCCTTCCTG TTTTGTCTCA CCCAGAAACG  
5761 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG  
5821 GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTTCGCCCCG AAGAACGTTT TCCAATGATG  
5881 AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTATTGACGC CGGGCAAGAG-

FIGURE 32C

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5941 CAACTCGGTC GCCGCATACA CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA  
6001 GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG  
6061 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC  
6121 GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG  
6181 AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG  
6241 TTGCGCAAAC TATTAAGTGG CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC  
6301 TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG  
6361 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG  
6421 GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT  
6481 ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA  
6541 CTGTCAGACC AAGTTTACTC ATATATACTT TAGATTGATT TAAAACTTCA TTTTAAATTT  
6601 AAAAGGATCT AGGTGAAGAT CCTTTTGTAT AATCTCATGA CCAAAATCCC TTAACGTGAG  
6661 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT  
6721 TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGTGCTT  
6781 TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG  
6841 CAGATACCAA ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT  
6901 GTAGCACC GC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC  
6961 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG  
7021 TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA  
7081 CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG  
7141 GACAGGTATC CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG  
7201 GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCTGA  
7261 TTTTGTGAT GCTCGTCA

FIGURE 32D

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Figure 33A1

pDEST13

Native protein in E. coli:  $\lambda$ PL  
promoter

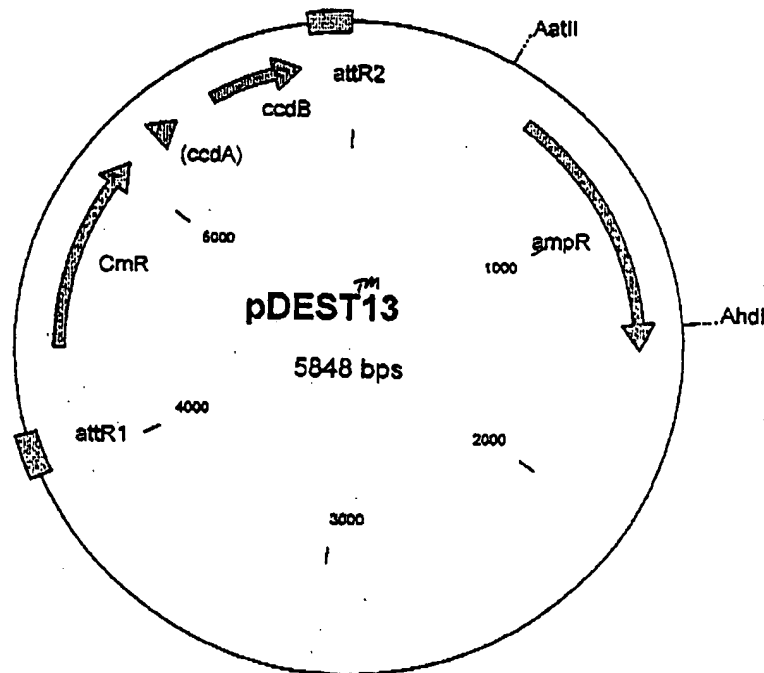
3721 tgggcaaacc aagacagcta aagatctctc acctaccaaa caatgcccc ctgcaaaaaa  
 acccgtttgg ttctgtcgat ttctagagag tggatggttt gttacggggg gacgtttttt

3781 taaattcata taaaaaacat acagataacc atctgcggtg ataaattatc tctggcggtg  
 atttaagtat attttttgta tgtctattgg tagacgccac tatttaatag agaccgccac

3841 ttgacataaa taccactggc ggtgatactg agcacatcag caggacgcac tgaccaccat  
aactgtattt atggtgaccg ccactatgac tctgtagtc gtcctgcgtg actggtggta

3901 gaaggtgacg ctcttaaaaa ttaagecctg agaaagggca gcattcaaag cagaaggctt  
 cttccactgc gagaattttt aattcgggac ctcttccgt cgtaagtttc gtcttcgaa

3961 tggggtgtgt gatacgaac gaagcattgg gatcatcaca agtttgtaca aaaaagctga  
 accccacaca ctatgctttg cttcgtaacc ctatagtggt tcaaacatgt tcttcgact



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## pDEST13 5848 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
599..1458		ampR
4123..3998		attR1
4372..5031		CmR
5151..5235		inactivated ccdA
5373..5678		ccdB
5719..5843		attR2
1 TTCACTGGCC GTCGTTTAC AACGTCGTGA CTGGGAAAAC CCTGGCGTTA CCCAACTTAA		
61 TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCGTAAT AGCGAAGAGG CCCGCACCGA		
121 TCGCCCTTCC CAACAGTTGC GCAGCCTGAA TGGCGAATGG CGCCTGATGC GGTATTTTCT		
181 CCTTACGCAT CTGTGCGGTA TTTCACACCG CATATGGTGC ACTCTCAGTA CAATCTGCTC		
241 TGATGCCGCA TAGTTAAGCC AGCCCCGACA CCCGCCAACA CCCGCTGACG CGCCCTGACG		
301 GGCTTGCTCTG CTCCCGGCAT CCGCTTACAG ACAAGCTGTG ACCGTCTCCG GGAGCTGCAT		
361 GTGTCAGAGG TTTTCACCGT CATCACCGAA ACGCGCGAGA CGAAAGGGCC TCGTGATACG		
421 CCTATTTTAA TAGGTTAATG TCATGATAAT AATGGTTTCT TAGACGTCAG GTGGCACTTT		
481 TCGGGGAAAT GTGCGCGGAA CCCCTATTTC TTTATTTTTC TAAATACATT CAAATATGTA		
541 TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT		
601 GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT		
661 TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG		
721 AGTGGGTTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA		
781 AGAACGTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG		
841 TATTGACGCC GGGCAAGAGC AACTCGGTGC CCGCATACAC TATTCTCAGA ATGACTTGGT		
901 TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG		
961 CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG		
1021 AGGACCGAAG GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCTTGA		
1081 TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC		
1141 TGTAGCAATG GCAACAACGT TGCGCAAACCT ATTAACCTGGC GAACTACTTA CTCTAGCTTC		
1201 CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC		
1261 GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG		
1321 CGGTATCATT GCAGCACTGG GGCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC		
1381 GACGGGGAGT CAGGCACTA TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCTTC		
1441 ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT		
1501 AAAAATTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC		
1561 CAAAATCCCT TAACGTGAGT TTTCTGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA		
1621 AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAACC		
1681 ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT		
1741 AACTGGCTTC AGCAGAGCGC AGATAACAAA TACTGTTCTT CTAGTGATAGC CGTAGTTAGG		
1801 CCACCACTTC AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC		
1861 AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT		
1921 ACCGGATAAG GCGCAGCGGT CCGGCTGAAC GGGGGGTTCC TGCACACAGC CCAGCTTGGA		
1981 GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT		
2041 TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG		
2101 CACGAGGGAG CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTCG GGTTCGCCA		
2161 CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA		
2221 CGCCAGCAAC GCGGCCTTTT TACGGTTCCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT		
2281 CTTTCTGCG TTATCCCTCG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA		
2341 TACCCTCGC CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA		
2401 GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA		
2461 CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTAGCT		
2521 CACTCATTAG GCACCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT TGTGTGGAAT		
2581 TGTGAGCGGA TAACAATTTT ACACAGGAAA CAGCTATGAC CATGATTACG CCAAGCTTGG		
2641 CTGCAGGTGA TGATTATCAG CCAGCAGAGA TTAAGGAAAA CAGACAGGTT TATTGAGCGC		
2701 TTATCTTTCC CTTTATTTTT GCTGCGGTAA GTCGCATAAA AACCATTCTT CATAATTCAA-		

FIGURE 33B

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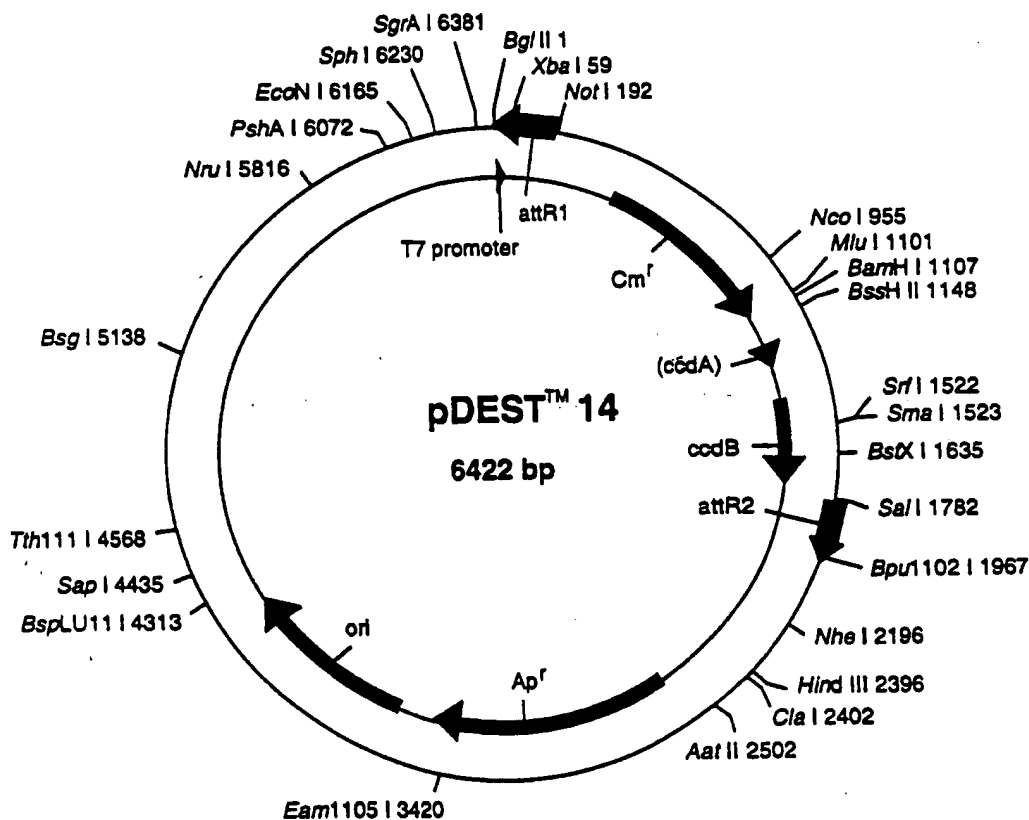
2761 TCCATTACT ATGTTATGTT CTGAGGGGAG TGAAAATTCC CCTAATTCGA TGAAGATTCT  
2821 TGCTCAATTG TTATCAGCTA TGCGCCGACC AGAACACCTT GCCGATCAGC CAAACGTCTC  
2881 TTCAGGCCAC TGA CTAGCGA TAACTTTCCC CACAACGGAA CAACTCTCAT TGCATGGGAT  
2941 CATTGGGTAC TGTGGGTTTA GTGGTTGTAA AAACACCTGA CCGCTATCCC TGATCAGTTT  
3001 CTTGAAGGTA AACTCATCAC CCCCAAGTCT GGCTATGCAG AAATCACCTG GCTCAACAGC  
3061 CTGCTCAGGG TCAACGAGAA TTAACATTCC GTCAGGAAAG CTTGGCTTGG AGCGTGTGG  
3121 TGCGGTCATG GAATTACCTT CAACCTCAAG CCAGAATGCA GAATCACTGG CTTTTTTGGT  
3181 TGTGCTTACC CATCTCTCCG CATCACCTTT GGTAAAGGTT CTAAGCTTAG GTGAGAACAT  
3241 CCCTGCCTGA ACATGAGAAA AAACAGGGTA CTCATACTCA CTTCTAAGTG ACGGCTGCAT  
3301 ACTAACCGCT TCATACATCT CGTAGATTTT TCTGGCGATT GAAGGGCTAA ATTCTTCAAC  
3361 GCTAACTTTG AGAATTTTTG CAAGCAATGC GGC GTTATAA GCATTTAATG CATTGATGCC  
3421 ATTAAATAAA GCACCAACGC CTGACTGCCC CATCCCCATC TTGTCTGCGA CAGATTCTTG  
3481 GGATAAGCCA AGTTCAATTT TCTTTTTTTT ATAAATTGCT TTAAGGCGAC GTGCGTCTC  
3541 AAGCTGCTCT TGTGTTAATG GTTTCTTTTT TGTGCTCATA CGTTAAATCT ATCACCGCAA  
3601 GGGATAAATA TCTAACACCG TGCGTGTGTA CTATTTTACC TCTGGCGGTG ATAATGGTTG  
3661 CATGTACTAA GGAGGTTGTA TGGAACAACG CATAACCTG AAAGATTATG CAATGCGCTT  
3721 TGGGCAAACC AAGACAGCTA AAGATCTCTC ACCTACCAA CAATGCCCCC CTGCAAAAAA  
3781 TAAATTCATA TAAAAACAT ACAGATAACC ATCTGCGGTG ATAAATTATC TCTGGCGGTG  
3841 TTGACATAAA TACCACCTGC GGTGATACTG AGCACATCAG CAGGACGCAC TGACCACCAT  
3901 GAAGGTGACG CTCTTAAAAA TTAAGCCCTG AAGAAGGGCA GCATTCAAAG CAGAAGGCTT  
3961 TGGGGTGTGT GATACGAAAC GAAGCATTGG GATCATCACA AGTTTGTACA AAAAAGCTGA  
4021 ACGAGAAACG TAAATGATA TAAATATCAA TATATTAAAT TAGATTTTGC ATAAAAACA  
4081 GACTACATAA TACTGTAAAA CACAACATAT CCAGTCACTA TGGCGGCCGC TAAGTTGGCA  
4141 GCATCACCCG ACGCACTTTG CGCCGAATAA ATACCTGTGA CGGAAGATCA CTTGCGAGAA  
4201 TAAATAAATC CTGGTGTCCC TGTTGATACC GGGAAAGCCCT GGGCCAACCT TTGGCGAAAA  
4261 TGAGACGTTG ATCGGCACGT AAGAGGTTCC AACTTTTACC ATAATGAAAT AAGATCACTA  
4321 CCGGGCGTAT TTTTGTAGTT ATCGAGATT TCAGGAGCTA AGGAAGCTAA AATGGAGAAA  
4381 AAAATCACTG GATATACCAC CGTTGATATA TCCCAATGGC ATCGTAAAGA ACATTTTGAG  
4441 GCATTTCACTG CAGTTGCTCA ATGTACCTAT AACCAGACCG TTCAGCTGGA TATTACGGCC  
4501 TTTTAAAGA CCGTAAAGAA AAATAAGCAC AAGTTTATC CGGCCTTTAT TCACATTCTT  
4561 GCCCGCCTGA TGAATGCTCA TCCGGAATTC CGTATGGCAA TGAAAGACGG TGAGCTGGTG  
4621 ATATGGGATA GTGTTCAACC TTGTTACACC GTTTTCCATG AGCAAACCTGA AACGTTTTCA  
4681 TCGCTCTGGA GTGAATACCA CGACGATTTC CGGCAGTTTC TACACATATA TTCGCAAGAT  
4741 GTGGCGTGTT ACGGTGAAAA CCTGGCCTAT TTCCCTAAAG GGTTTATTGA GAATATGTTT  
4801 TTCGTCTCAG CCAATCCCTG GGTGAGTTTC ACCAGTTTGT ATTTAAACGT GGCCAATATG  
4861 GACAACCTCT TCGCCCCCGT TTTCACCATG GGCAAATATT ATACGCAAGG CGACAAGGTG  
4921 CTGATGCCGC TGGCGATTCA GGTTCATCAT GCCGTCTGTG ATGGCTTCCA TGTGCGCAGA  
4981 ATGCTTAATG AATTACAACA GTACTGCGAT GAGTGGCAGG GCGGGGCGTA AACCGTGGA  
5041 TCCGGCTTAC TAAAAGCCAG ATAACAGTAT GCGTATTTGC GCGCTGATTT TTGCGGTATA  
5101 AGAATATATA CTGATATGTA TACCCGAAGT ATGTCAAAAA GAGGTGTGCT ATGAAGCAGC  
5161 GTATTACAGT GACAGTTGAC AGCGACAGCT ATCAGTTGCT CAAGGCATAT ATGATGTCAA  
5221 TATCTCCGGT CTGGTAAGCA CAACCATGCA GAATGAAGCC CGTCGTCTGC GTGCCGAACG  
5281 CTGGAAGCG GAAAATCAGG AAGGGATGGC TGAGGTCGCC CGGTTTATTG AAATGAACGG  
5341 CTCTTTTGCT GACGAGAACA GGGACTGTG AAATGCAGTT TAAGGTTTAC ACCTATAAAA  
5401 GAGAGAGCCG TTATCGTCTG TTTGTGGATG TACAGAGTGA TATTATTGAC ACGCCCGGGC  
5461 GACGGATGGT GATCCCCCTG GCCAGTGCAC GTCTGCTGTC AGATAAAGTC TCCCGTGAAC  
5521 TTTACCCGGT GGTGCATATC GGGGATGAAA GCTGGCGCAT GATGACCACC GATATGCGCA  
5581 GTGTGCCGGT CTCCGTTATC GGGGAAGAAG TGGCTGATCT CAGCCACCGC GAAAATGACA  
5641 TCAAAAACGC CATTAACTG ATGTTCTGGG GAATATAAAT GTCAGGCTCC GTTATACACA  
5701 GCCAGTCTGC AGGTCGACCA TAGTGACTGG ATATGTTGTG TTTTACAGTA TTATGTAGTC  
5761 TGTTTTTTAT GCAAAATCTA ATTTAATATA TTGATATTTA TATCATTTTA CGTTTCTCGT  
5821 TCAGCTTTCT TGTACAAAGT GGTGATAA

FIGURE 33C

Figure 34A: pDEST14 Native Protein Expression in *E. coli*, T7 Promoter

3961 tgccggccac gatgcgtccg gcgtagagga tcgagatctc gatcccgcca aattaatagc  
 acggccggtg ctacgcaggc cgcattctct agctctagag ctaggcgct ttaattatgc  
 4021 // actcactata gggagaccac aacggtttcc ctctagatca caagtttcta caaaaaagct  
 // tgagtgatat ccctctggtg ttgccaaagg gagatctagt gttcaaacat gttttttcga //

Handwritten annotations above the sequence include: *Bgl* II, *Aat* I, *PT* I, *Xba* I, *Eco* RI, *Sma* I, *Bst* I, *Bgl* II, *Xba* I, *Eco* RI, *Sma* I, *Bst* I.



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## pDEST14 6422 bp (rotated to position 4000)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
185..61		attR1
435..1094		CmR
1214..1298		inactivated ccdA
1436..1741		ccdB
1782..1906		attR2
2632..3489		ampR
1	CGATCCCGCG AAATTAATAC GACTCACTAT AGGGAGACCA CAACGGTTTC CCTCTAGATC	
61	ACAAGTTTGT AAAAAAAGC TGAACGAGAA ACGTAAATG ATATAAATAT CAATATATTA	
121	AATTAGATTT TGCATAAAAA ACAGACTACA TAATACTGTA AAACACAACA TATCCAGTCA	
181	CTATGGCGGC CGCTAAGTTG GCAGCATCAC CCGACGCACT TTGCGCCGAA TAAATACCTG	
241	TGACGGAAGA TCACTTCGCA GAATAAATAA ATCCTGGTGT CCCTGTTGAT ACCGGGAAGC	
301	CCTGGGCCAA CTTTGGCGA AAATGAGACG TTGATCGGCA CGTAAGAGGT TCCAACTTTC	
361	ACCATAATGA AATAAGATCA CTACCGGGCG TATTTTTTGA GTTATCGAGA TTTTCAGGAG	
421	CTAAGGAAGC TAAAATGGAG AAAAAATCA CTGGATATAC CACCGTTGAT ATATCCCAAT	
481	GGCATCGTAA AGAACATTTT GAGGCATTTT AGTCAGTTGC TCAATGTACC TATAACCAGA	
541	CCGTTTCAGCT GGATATTACG GCCTTTTTAA AGACCGTAAA GAAAAATAAG CACAAGTTTT	
601	ATCCGGCCTT TATTCACATT CTTGCCCGCC TGATGAATGC TCATCCGGAA TTCCGTATGG	
661	CAATGAAAGA CCGTGAGCTG GTGATATGGG ATAGTGTTCA CCCTTGTTAC ACCGTTTTCC	
721	ATGAGCAAAC TGAAACGTTT TCATCGCTCT GGAGTGAATA CCACGACGAT TTCCGGCAGT	
781	TTCTACACAT ATATTCGCAA GATGTGGCGT GTTACGGTGA AAACCTGGCC TATTTCCCTA	
841	AAGGGTTTAT TGAGAATATG TTTTTCGTCT CAGCCAATCC CTGGGTGAGT TTCACAGTT	
901	TTGATTTTAA CGTGGCCAAT ATGGACAACT TCTTCGCCCC CGTTTTTCACC ATGGGCAAAAT	
961	ATTATACGCA AGGCGACAAG GTGCTGATGC CGCTGGCGAT TCAGGTTTAT CATGCCGTCT	
1021	GTGATCGCTT CCATGTCCGC AGAATGCTTA ATGAATTACA ACAGTACTGC GATGATGGC	
1081	AGGGCGGGGC GTAAACGCGT GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTATT	
1141	TGCGCGCTGA TTTTTCGGGT ATAAGAATAT ATACTGATAT GTATACCCGA AGTATGTCAA	
1201	AAAGAGGTGT GCTATGAAGC AGCGTATTAC AGTGACAGTT GACAGCGACA GCTATCAGTT	
1261	GCTCAAGGCA TATATGATGT CAATATCTCC GGTCTGGTAA GCACAACCAT GCAGAATGAA	
1321	GCCCGTCGTC TGCGTGCCGA ACGCTGGAAG GCGGAAATC AGGAAGGGAT GGCTGAGGTC	
1381	GCCCGGTTTA TTGAAATGAA CGGCTCTTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA	
1441	GTTTAAGGTT TACACCTATA AAAGAGAGAG CCGTTATCGT CTGTTTGTGG ATGTACAGAG	
1501	TGATATTATT GACACGCCCC GCGCAGCGAT GGTGATCCCC CTGGCCAGTG CACGCTCTGCT	
1561	GTCAGATAAA GTCTCCCGTG AACTTTACCC GGTGGTGCAT ATCGGGGATG AAAGCTGGCG	
1621	CATGATGACC ACCGATATGG CCAGTGTGCC GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA	
1681	TCTCAGCCAC CGCGAAAATG ACATCAAAAA CGCCATTAAC CTGATGTTCT GGGGAATATA	
1741	AATGTCAGGC TCCCTTATAC ACAGCCAGTC TGCAGGTCGA CCATAGTGAC TGGATATGTT	
1801	GTGTTTTACA GTATTATGTA GTCTGTTTTT TATGCAAAAT CTAATTTAAT ATATTGATAT	
1861	TTATATCATT TTACGTTTCT CGTTCAGCTT TCTTGTAACA AGTGGTGATG ATCCGGCTGC	
1921	TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA	
1981	ACCCCTTGGG GCCTCTAAAC GGGTCTTGAG GGGTTTTTTG CTGAAAGGAG GAACTATATC	
2041	CGGATATCCA CAGGACGGGT GTGGTCGCCA TGATCGCGTA GTCGATAGTG GCTCCAAGTA	
2101	GCGAAGCGAG CAGGACTGGG CCGCGGCCAA AGCGGTCGGA CAGTGCTCCG AGAACGGGTG	
2161	CGCATAGAAA TTGCATCAAC GCATATAGCG CTAGCAGCAC GCCATAGTGA CTGGCGATGC	
2221	TGTCCGAATG GACGATATCC CGCAAGAGGC CCGGCAGTAC CGGCATAACC AAGCCTATGC	
2281	CTACAGCATC CAGGGTGACG GTGCCGAGGA TGACGATGAG CGCATTGTTA GATTTTCATAC	
2341	ACGGTGCCTG ACTGCGTTAG CAATTTAACT GTGATAAACT ACCGCATTAA AGCTTATCGA	
2401	TGATAAGCTG TCAAAACATGA GAATCTTGA AGACGAAAGG GCCTCGTGAT ACGCCTATTT	
2461	TTATAGGTTA ATGTCATGAT AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA	
2521	AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC	
2581	ATGAGACAAT AACCCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT	
2641	CAACATTTCC GTGTGCCCC TATTCCTTTT TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT	
2701	CACCCAGAAA CGCTGGTGAA AGTAAAGAT GCTGAAGATC AGTTGGGTGC ACCAGTGGGT-	

FIGURE 34B



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2761 TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT  
2821 TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CCGTATTATC CCGTGTGAC  
2881 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTGTAGTAC  
2941 TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT ATGCAGTGCT  
3001 GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG  
3061 AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG  
3121 GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCTGCAGCA  
3181 ATGGCAACAA CGTTGCGCAA ACTATTAAC TGGCAACTAC TTAATCTAGC TTCCCGGCAA  
3241 CAATTAATAG ACTGGATGGA GCGGATAAA GTTGCAGGAC CACTTCTGCG CTCGGCCCTT  
3301 CCGGCTGGCT GGTATTATGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC  
3361 ATTGCAGCAC TGGGGCCAGA TGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG  
3421 AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT  
3481 AAGCATTTGT AACTGTCTGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAACTT  
3541 CATTTTTTAA TTAAGAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT GACCAAAATC  
3601 CCTTAACGTG AGTTTTTCGT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT  
3661 TCTTGAGATC CTTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA  
3721 CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTCCGAA GGTAACGGC  
3781 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC  
3841 TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT  
3901 GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACGGAT  
3961 AAGGCGCAGC GGTGCGGCTG AACGGGGGGT TCGTGACAC AGCCAGCTT GGAGCGAACG  
4021 ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA  
4081 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG  
4141 GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG CCACCTCTGA  
4201 CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC  
4261 AACGCGGCCT TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT  
4321 GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT  
4381 CGCCGAGCG GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCTG  
4441 ATGCGGTATT TTCTCCTTAC GCATCTGTGC GGTATTTTAC ACCGCATATA TGGTGCACCTC  
4501 TCAGTACAAT CTGCTCTGAT GCCGCATAGT TAAGCCAGTA TACACTCCGC TATCGCTACG  
4561 TGACTGGGTC ATGGCTGCGC CCCGACACCC GCCAACACCC GCTGACGCGC CCTGACGGGC  
4621 TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC GTCTCCGGGA GCTGCATGTG  
4681 TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGAGGCGA CTGCGGTAA GCTCATCAGC  
4741 GTGGTCGTGA AGCGATTAC AGATGTCTGC CTGTTTCATC CCGTCCAGCT CGTTGAGTTT  
4801 CTCCAGAAGC GTTAATGTCT GGCTTCTGAT AAAGCGGGCC ATGTTAAGGG CGGTTTTTTT  
4861 CTGTTTGGTC ACTGATGCCT CCGTGTAAAG GGGATTCTG TTGATGGGG TAATGATACC  
4921 GATGAAACGA GAGAGGATGC TCACGATACG GGTACTGAT GATGAACATG CCCGGTTACT  
4981 GGAACGTTGT GAGGGTAAAC AACTGGCGGT ATGGATGCGG CGGGACCAGA GAAAAATCAC  
5041 TCAGGGTCAA TGCCAGCGCT TCGTTAATAC AGATGTAGGT GTTCCACAGG GTAGCCAGCA  
5101 GCATCTGCG ATGACAGATCC GGAACATAAT GGTGCAGGGC GCTGACTTCC GCGTTTCCAG  
5161 ACTTTACGAA ACACGGAAAC CGAAGACCAT TCATGTTGTT GCTCAGGTCG CAGACGTTTT  
5221 GCAGCAGCAG TCGCTTCACG TTCGCTCGCG TATCGGTGAT TCATTCTGCT AACCAGTAAG  
5281 GCAACCCCGC CAGCCTAGCC GGGTCTCTCA CGACAGGAGC ACGATCATGC GCACCCGTGG  
5341 CCAGGACCCA ACGCTGCCCG AGATGCGCCG CGTGCGGCTG CTGGAGATGG CGGACGCGAT  
5401 GGATATGTTT TGCCAAGGGT TGGTTTGCGC ATTACAGTTC CTCCGCAAGA ATTGATTGGC  
5461 TCCAATTCTT GGAGTGGTGA ATCCGTTAGC GAGGTGCCGC CCGCTTCCAT TCAGGTGAGG  
5521 GTGGCCCGGC TCCATGCACC GCGACGCAAC GCGGGGAGGC AGACAAGGTA TAGGGCGGGC  
5581 CCTACAATCC ATGCCAACCC GTTCCATGTG CTCGCCGAGG CGGCATAAAT CCGCGTGACG  
5641 ATCAGCGGTC CAGTGATCGA AGTTAGGCTG GTAAGAGCCG CGAGCGATCC TTGAAGCTGT  
5701 CCCTGATGGT CGTCATCTAC CTGCCTGGAC AGCATGGCCT GCAACGCGGG CATCCCGATG  
5761 CCGCCGGAAG CGAGAAGAAT CATAATGGGG AAGGCCATCC AGCCTCGCGT CGCGAACGCC  
5821 AGCAAGACGT AGCCACGCGC GTCGGCCGCC ATGCCGCGCA TAATGGCCTG CTCTCGCCG  
5881 AAACGTTTGG TGGCGGGACC AGTGACGAAG GCTTGAGCGA GGGCGTGCAA GATTCCGAAT  
5941 ACCGCAAGCG ACAGGCCGAT CATCGTCGCG CTCCAGCGAA AGCGGTCTCT GCCGAAAATG  
6001 ACCCAGAGCG CTGCCGGCAC CTGTCTTACG AGTTGCATGA TAAAGAAGAC AGTCATAAGT  
6061 GCGGCGACGA TAGTCATGCC CCGCGCCAC CGGAAGGAGC TGAAGGGTT GAAGGCTCTC  
6121 AAGGGCATCG GTCGATCGAC GCTCTCCCTT ATGCGACTCC TGCATTAGGA AGCAGCCCAG  
6181 TAGTAGGTTG AGGCCGTTGA GCACCGCCGC CGCAAGGAAT GGTGCATGCA AGGAGATGGC

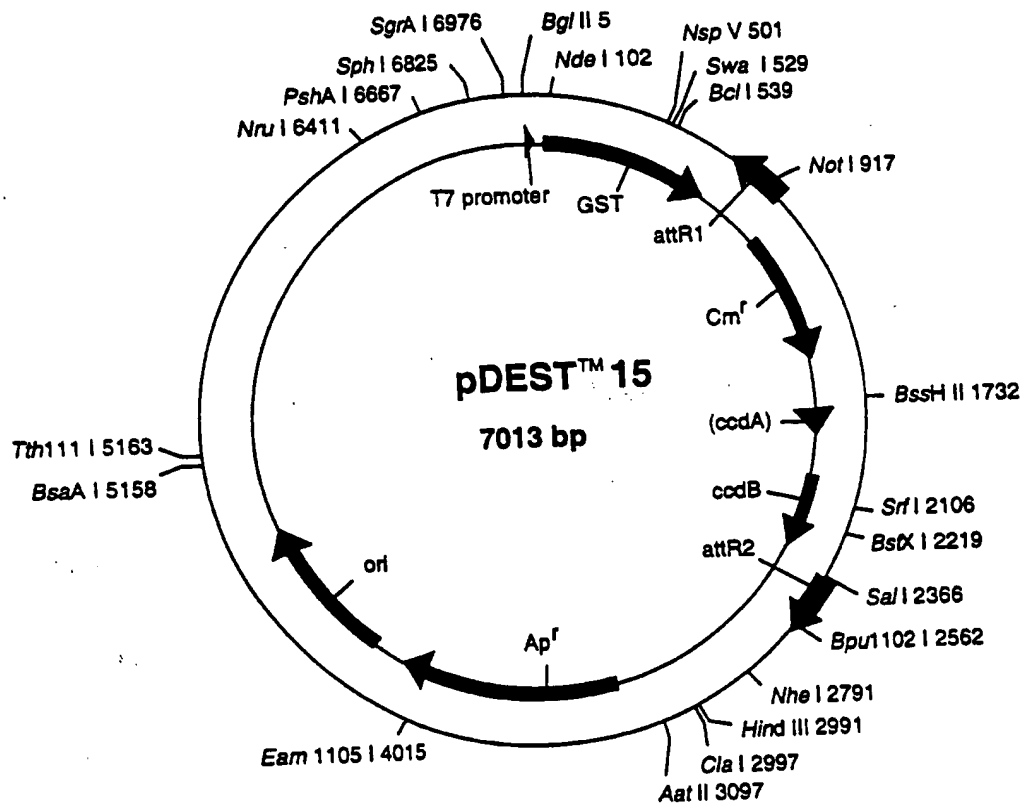
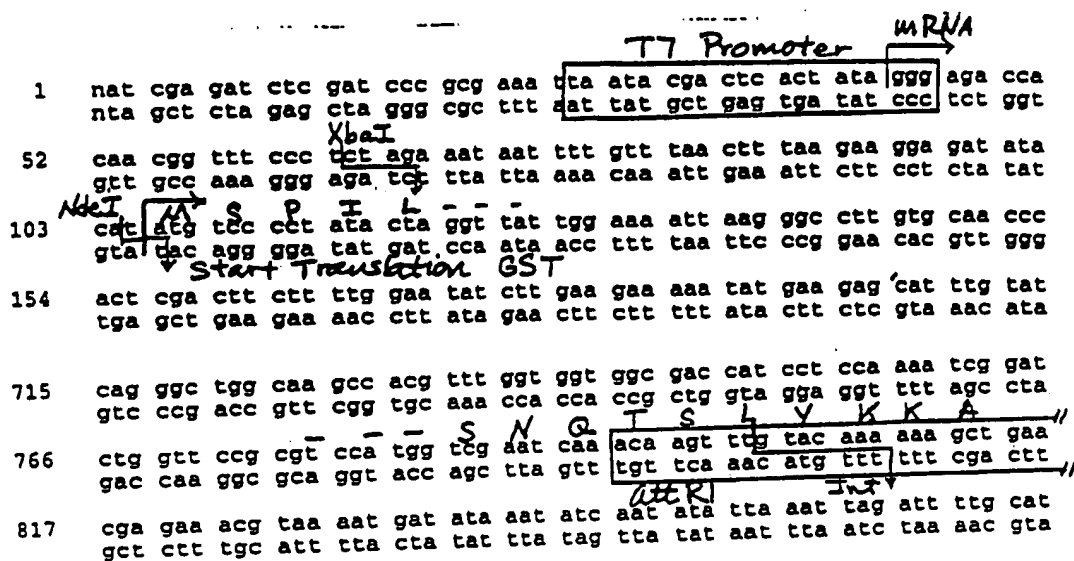
FIGURE 34C

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6241 GCCCAACAGT CCCCCGGCCA CGGGGCCTGC CACCATACCC ACGCCGAAAC AAGCGCTCAT  
6301 GAGCCCGAAG TGGCGAGCCC GATCTTCCCC ATCGGTGATG TCGGCGATAT AGGCGCCAGC  
6361 AACCGCACCT GTGGCGCCGG TGATGCCGGC CACGATGCGT CCGGCGTAGA GGATCGAGAT  
6421 CT

FIGURE 34D

**Figure 35A: pDEST15 Glutathione-S-transferase Fusion in *E. coli*, T7 Promoter**



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## pDEST15 7013 bp

<u>Location (Base Nos.)</u>			<u>Gene Encoded</u>			
	108..776		GST			
	916..792		attR1			
	1025..1537		CmR			
	1804..1888		inactivated ccdA			
	2026..2331		ccdB			
	2372..2496		attR2			
	3233..4093		ampR			
1	ATCGAGATCT	CGATCCCGCG	AAATTAATAC	GACTCACTAT	AGGGAGACCA	CAACGGTTTC
61	CCTCTAGAAA	TAATTTTGTT	TAACTTTAAG	AAGGAGATAT	ACATATGTCC	CCTATACTAG
121	GTTATTGGAA	AATTAAGGGC	CTTGTGCAAC	CCACTCGACT	TCTTTTGGAA	TATCTTGAAG
181	AAAAATATGA	AGAGCATTTG	TATGAGCGCG	ATGAAGGTGA	TAAATGGCGA	AACAAAAAGT
241	TTGAATTGGG	TTTGGAGTTT	CCCAATCTTC	CTTATTATAT	TGATGGTGAT	GTTAAATTAA
301	CACAGTCTAT	GGCCATCATA	CGTTATATAG	CTGACAAGCA	CAACATGTTG	GGTGGTTGTC
361	CAAAAGAGCG	TGCAGAGATT	TCAATGCTTG	AAGGAGCGGT	TTTGGATATT	AGATACGGTG
421	TTTCGAGAAT	TGCATATAGT	AAAGACTTTG	AAACTCTCAA	AGTTGATTTT	CTTAGCAAGC
481	TACCTGAAAT	GCTGAAAATG	TTCGAAGATC	GTTTATGTCA	TAAAACATAT	TTAAATGGTG
541	ATCATGTAAC	CCATCCTGAC	TTTATGTTGT	ATGACGCTCT	TGATGTTGTT	TTATACATGG
601	ACCCAATGTG	CCTGGATGCG	TTCCCAAAT	TAGTTTGTGT	TAAAAAACGT	ATTGAAGCTA
661	TCCCACAAAT	TGATAAGTAC	TTGAAATCCA	GCAAGTATAT	AGCATGGCCT	TTGCAGGGCT
721	GGCAAGCCAC	GTTTGGTGGT	GGCGACCATC	CTCCAAATC	GGATCTGGTT	CCGCGTCCAT
781	GGTCGAATCA	AACAAGTTTG	TACAAAAAAG	CTGAACGAGA	AACGTAAAT	GATATAAATA
841	TCAATATATT	AAATTAGATT	TTGCATAAAA	AACAGACTAC	ATAATACTGT	AAAACACAAC
901	ATATCCAGTC	ACTATGGCGG	CCGCATTAGG	CACCCAGGC	TTTACACTTT	ATGCTTCCGG
961	CTCGTATAAT	GTGTGGATTT	TGAGTTAGGA	TCCGTCGAGA	TTTTCAGGAG	CTAAGGAAGC
1021	TAAAATGGAG	AAAAAAATCA	CTGGATATAC	CACCGTTGAT	ATATCCCAAT	GGCATCGTAA
1081	AGAACATTTT	GAGGCATTTT	AGTCAGTTGC	TCAATGTACC	TATAACCAGA	CCGTTTCAAGT
1141	GGATATTACG	GCCTTTTTTA	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT	ATCCGGCCCT
1201	TATTCACATT	CTTGCCCGCC	TGATGAATGC	TCATCCGGAA	TTCCGTATGG	CAATGAAAGA
1261	CGGTGAGCTG	GTGATATGGG	ATAGTGTTCA	CCCTTGTTAC	ACCGTTTTCC	ATGAGCAAAC
1321	TGAAACGTTT	TCATCGCTCT	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT	TTCTACACAT
1381	ATATTGCGAA	GATGTGGCGT	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA	AAGGGTTTAT
1441	TGAGAATATG	TTTTTCGTCT	CAGCCAATCC	CTGGGTGAGT	TTTACCAGTT	TTGATTTAAA
1501	CGTGGCCAAT	ATGGACAAC	TCTTCGCCCC	CGTTTTTACC	ATGGGCAAAT	ATTATACGCA
1561	AGGCGACAAG	GTGCTGATGC	CGCTGGCGAT	TCAGGTTTCA	CATGCCGTCT	GTGATGGCTT
1621	CCATGTCGGC	AGAATGCTTA	ATGAATTACA	ACAGTACTGC	GATGAGTGGC	AGGGCGGGGC
1681	GTAATCTAGA	GGATCCGGCT	TACTAAAAGC	CAGATAACAG	TATGCGTATT	TGCGCGCTGA
1741	TTTTTGCGGT	ATAAGAATAT	ATACTGATAT	GTATACCCGA	AGTATGTCAA	AAAGAGGTGT
1801	GCTATGAAGC	AGCGTATTAC	AGTGACAGTT	GACAGCGACA	GCTATCAGTT	GCTCAAGGCA
1861	TATATGATGT	CAATATCTCC	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA	GCCCCGTCGT
1921	TGCGTGCCGA	ACGCTGGAAA	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC	GCCCCGTTTA
1981	TTGAAATGAA	CGGCTCTTTT	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA	GTTTAAGGTT
2041	TACACCTATA	AAAGAGAGAG	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG	TGATATTATT
2101	GACACGCCCC	GGCGACGGAT	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT	GTCAGATAAA
2161	GTCTCCCGTG	AACTTTACCC	GGTGGTGCAT	ATCGGGGATG	AAAGCTGGCG	CATGATGACC
2221	ACCGATATGG	CCAGTGTGCC	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA	TCTCAGCCAC
2281	CGCGAAAATG	ACATCAAAAA	CGCCATTAA	CTGATGTTCT	GGGGAATATA	AATGTCAGGC
2341	TCCCTTATAC	ACAGCCAGTC	TGCAGGTCGA	CCATAGTGAC	TGGATATGTT	GTGTTTTACA
2401	GTATTATGTA	GTCTGTTTTT	TATGCAAAAT	CTAATTTAAT	ATATTGATAT	TTATATCATT
2461	TTACGTTTCT	CGTTCAGCTT	TCTTGTACAA	AGTGGTTTGA	TTCCAGCCCG	GATCCGGCTG
2521	CTAACAAAGC	CCGAAAGGAA	GCTGAGTTGG	CTGCTGCCAC	CGCTGAGCAA	TAAGTATGAT
2581	AACCCCTTGG	GGCCTCTAAA	CGGGTCTTGA	GGGGTTTTTT	GCTGAAAGGA	GGAACATATAT
2641	CCGGATATCC	ACAGGACGGG	TGTGGTCCGC	ATGATCGCGT	AGTCGATAGT	GGCTCCAAGT

Figure 35B

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2701 AGCGAAGCGA GCAGGACTGG GCGGCGGCCA AAGCGGTCCG ACAGTGCTCC GAGAACGGGT  
2761 GCGCATAGAA ATTGCATCAA CGCATATAGC GCTAGCAGCA CGCCATAGTG ACTGGCGATG  
2821 CTGTCCGAAT GGACGATATC CCGCAAGAGG CCCGGCAGTA CCGGCATAAC CAAGCCTATG  
2881 CCTACAGCAT CCAGGGTGAC GGTGCCGAGG ATGACGATGA GCGCATTGTT AGATTTCATA  
2941 CACGGTGCCT GACTGCGTTA GCAATTTAAC TGTGATAAAC TACCGCATT AAGCTTATCG  
3001 ATGATAAGCT GTCAAACATG AGAATTCCTG AAGACGAAAG GGCCTCGTGA TACGCCCTATT  
3061 TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG  
3121 AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT  
3181 CATGAGACAA TAACCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT  
3241 TCAACATTTC CGTGTGCCCC TTATTCCCTT TTTTGGCGCA TTTTGCCTTC CTGTTTTTGC  
3301 TCACCCAGAA ACGCTGGTGA AAGTAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG  
3361 TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG  
3421 TTTTCCAAATG ATGAGCACTT TTAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGTTGA  
3481 CGCCGGGCAA GAGCAACTCG GTCGCCGCGT ACACTATTCT CAGAATGACT TGGTTGAGTA  
3541 CTCACCGAGT ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC  
3601 TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC  
3661 GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG  
3721 GGAACCGGAG CTGAATGAAG CCATACCAA CGACGAGCGT GACACCACGA TGCCTGCAGC  
3781 AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA  
3841 ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT  
3901 TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT  
3961 CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG  
4021 GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT  
4081 TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAACT  
4141 TCATTTTTAA TTTAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT  
4201 CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC  
4261 TTCTTGAGAT CTTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT  
4321 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACCTG  
4381 CTTCAGCAGA GCGCAGATAC CAAATACTGT CTTTCTAGTG TAGCCGAGT TAGGCCACCA  
4441 CTTCAAGAAC TCTGTAGCAC CGCTACATA CTTGCTCTG CTAATCCTGT TACCACTGGC  
4501 TGCTGCCAGT GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA  
4561 TAAGGCGCAG CGGTCCGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC  
4621 GACCTACACC GAAGTGAAGT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA  
4681 AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCAGCAG  
4741 GGAGCTTCCA GGGGGAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG  
4801 ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG  
4861 CAACGCGGCC TTTTACGGT TTCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC  
4921 TCGTTATATCC CCGTATTCTG TGGATAAACG TATTACCGCC TTTGAGTGAG CTGATACCGC  
4981 TCGCCGCAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCT  
5041 GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAT ATGGTGCATC  
5101 CTCAGTACAA TCTGCTCTGA TGCCGCATAG TTAAGCCAGT ATACACTCCG CTATCGCTAC  
5161 GTGACTGGGT CATGGCTGCG CCCCAGACAC CGCCAACACC CGCTGACCGC CCCTGACGGG  
5221 CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT  
5281 GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA GCTGCGGTA AGCTCATCAG  
5341 CGTGGTCTGT AAGCGATTCA CAGATGTCTG CCGTTCATC CGCGTCCAGC TCGTTGAGTT  
5401 TCTCCAGAAG CGTTAATGTC TGGCTTCTGA TAAAGCGGGC CATGTTAAGG GCGGTTTTTT  
5461 CCTGTTTGGT CACTGATGCC TCCGTGTAAG GGGGATTTCT GTTCATGGGG GTAATGATAC  
5521 CGATGAAACG AGAGAGGATG CTCACGATAC GGGTTACTGA TGATGAACAT GCCCGGTTAC  
5581 TGGAACGTTG TGAGGGTAAA CAACTGGCGG TATGGATGCG GCGGGACCAG AGAAAAATCA  
5641 CTCAGGGTCA ATGCCAGCGC TTCGTTAATA CAGATGTAGG TGTTCCACAG GGTAGCCAGC  
5701 AGCATCCTGC GATGCAGATC CGGAACATAA TGGTGCAGGG CGCTGACTTC CGCGTTTCCA  
5761 GACTTTACGA AACACGGAAA CCGAAGACCA TTCATGTTGT TGCTCAGGTC CGCAGCGTTT  
5821 TGCAGCAGCA GTCGCTTAC GTTCGCTCGC GTATCGGTGA TTCATTCTGC TAACCAAGTAA  
5881 GGCAACCCCG CCAGCCTAGC CGGGTCTCA ACGACAGGAG CACGATCATG CGCACCCGTG  
5941 GCCAGGACCC AACGCTGCCC GAGATGCGCC GCGTGGGGCT GCTGGAGATG GCGGACGCGA  
6001 TGGATATGTT CTGCCAAGGG TTGGTTTGCG CATTCACAGT TCTCCGCAAG AATTGATTGG  
6061 CTCCAATTCT TGGAGTGGTG AATCCGTTAG CGAGGTGCCG CCGGCTTCCA TTCAGGTCGA  
6121 GGTGGCCCGG CTCCATGCAC CGCGACGCAA CGCGGGGAGG CAGACAAGGT ATAGGGCGCG-

FIGURE 35C

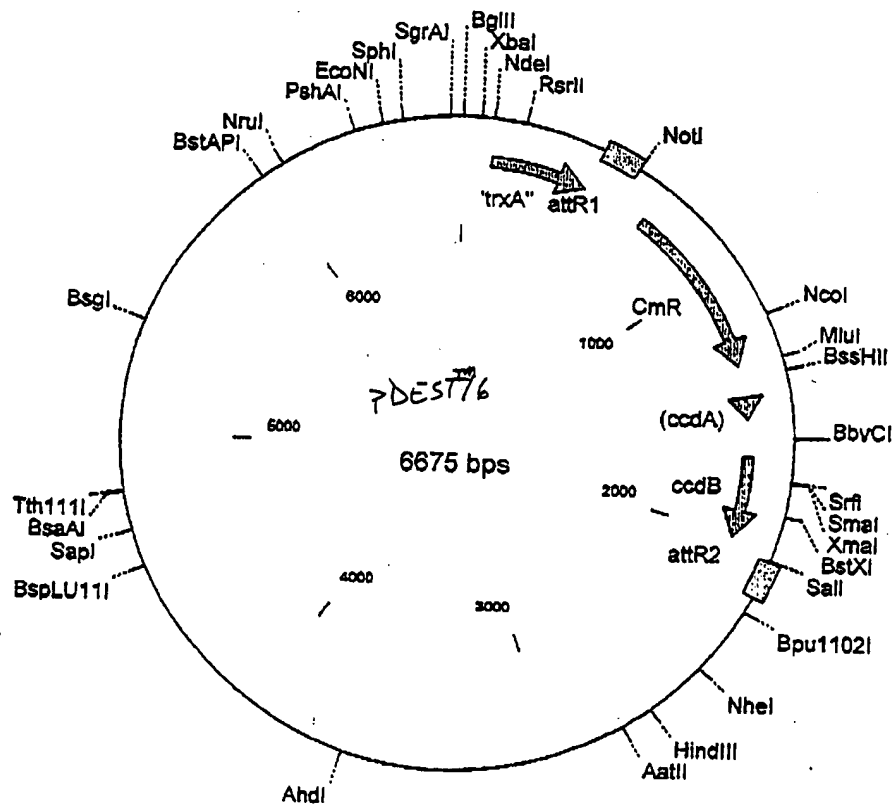
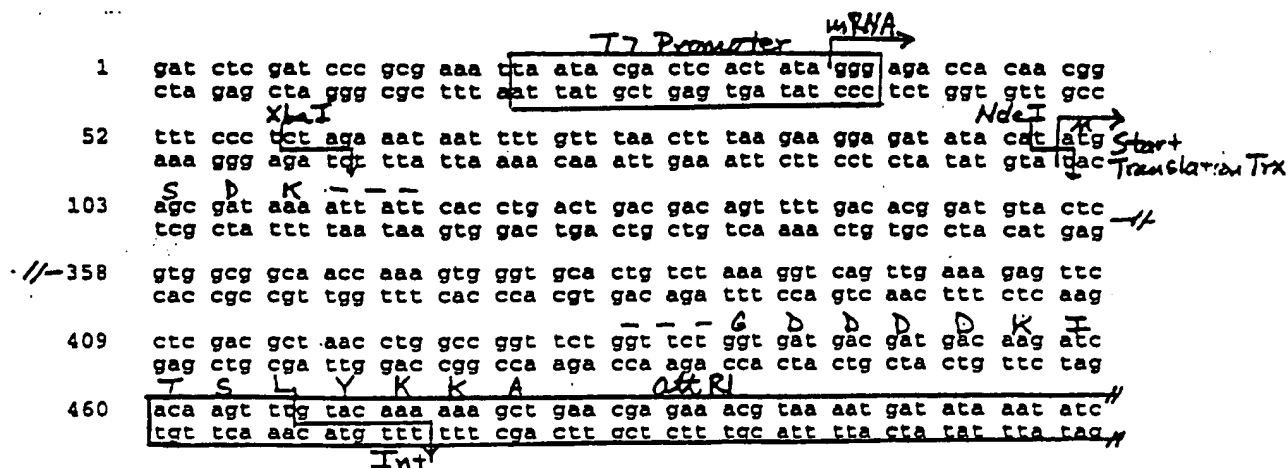
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6181 GCCTACAATC CATGCCAACC CGTTCCATGT GCTCGCCGAG GCGGCATAAA TCGCCGTGAC  
6241 GATCAGCGGT CCAGTGATCG AAGTTAGGCT GGTAAGAGCC GCGAGCGATC CTTGAAGCTG  
6301 TCCCTGATGG TCGTCATCTA CCTGCCTGGA CAGCATGGCC TGCAACGCGG GCATCCCGAT  
6361 GCCGCCGGAA GCGAGAAGAA TCATAATGGG GAAGGCCATC CAGCCTCGCG TCGCGAACGC  
6421 CAGCAAGACG TAGCCCAGCG CGTCGGCCGC CATGCCGGCG ATAATGGCCT GCTTCTCGCC  
6481 GAAACGTTTG GTGGCGGGAC CAGTGACGAA GGCTTGAGCG AGGGCGTGCA AGATTCCGA  
6541 TACCGCAAGC GACAGGCCGA TCATCGTCGC GCTCCAGCGA AAGCGGTCCT CGCCGAAAA  
6601 GACCCAGAGC GCTGCCGGCA CCTGTCCTAC GAGTTGCATG ATAAAGAAGA CAGTCATAAG  
6661 TGCGGCGACG ATAGTCATGC CCCGCGCCCA CCGGAAGGAG CTGACTGGGT TGAAGGCTCT  
6721 CAAGGGCATC GGTGATCGA CGCTCTCCCT TATGCGACTC CTGCATTAGG AAGCAGCCCA  
6781 GTAGTAGGTT GAGGCCGTTG AGCACCGCCG CCGCAAGGAA TGGTGCATGC AAGGAGATGG  
6841 CGCCCAACAG TCCCCCGGCC ACGGGGCCTG CCACCATACC CACGCCGAAA CAAGCGCTCA  
6901 TGAGCCCGAA GTGGCGAGCC CGATCTTCCC CATCGGTGAT GTCGGCGATA TAGGCGCCAG  
6961 CAACCGCACC TGTGGCGCCG GTGATGCCGG CCACGATGCG TCCGGCGTAG AGG

FIGURE 351)

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**Figure 36A:** *pDEST16* **Thioredoxin N-Fusion Protein in E. coli with T7 Promoter**



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## pDEST16 6675 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
104..457		trxA
585..461		attR1
694..1353		CmR
1473..1557		inactivated ccdA
1695..2000		ccdB
2041..2165		attR2

1	AGATCTCGAT	CCCGCGAAAT	TAATACGACT	CACTATAGGG	AGACCACAAC	GGTTTCCCTC
61	TAGAAATAAT	TTTGTTTAAC	TTTAAGAAGG	AGATATACAT	ATGAGCGATA	AAATTATTCA
121	CCTGACTGAC	GACAGTTTTG	ACACGGATGT	ACTCAAAGCG	GACGGGGCGA	TCCTCGTCGA
181	TTTCTGGGCA	GAGTGGTGCG	GTCCGTGCAA	AATGATCGCC	CCGATTCTGG	ATGAAATCGC
241	TGACGAATAT	CAGGGCAAAC	TGACCGTTGC	AAAACGAAC	ATCGATCAAA	ACCCTGGCAC
301	TGCGCCGAAA	TATGGCATCC	GTGGTATCCC	GACTCTGCTG	CTGTTCAAAA	ACGGTGAAGT
361	GGCGGCAACC	AAAGTGGGTG	CACTGTCTAA	AGGTCAGTTG	AAAGAGTTCC	TCGACGCTAA
421	CCTGGCCGGT	TCTGGTCTCT	GTGATGACGA	TGACAAGATC	ACAAGTTTGT	ACAAAAAAGC
481	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA	AATTAGATTT	TGCATAAAAA
541	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA	CTATGGCGGC	CGCATTAGGC
601	ACCCGAGGCT	TTACACTTTA	TGCTTCCGGC	TCGTATAATG	TGTGGATTTT	GAGTTAGGAT
661	CCGGCGAGAT	TTTCAGGAGC	TAAGGAAGCT	AAAATGGAGA	AAAAAATCAC	TGGATATACC
721	ACCGTTGATA	TATCCCAATG	GCATCGTAAA	GAACATTTTG	AGGCATTTCA	GTCAGTTGCT
781	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG	GATATTACGG	CCTTTTTTAA	GACCGTAAAG
841	AAAAATAAGC	ACAAGTTTTA	TCCGGCCTTT	ATTACATTTC	TTGCCCGCCT	GATGAATGCT
901	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC	GGTGAGCTGG	TGATATGGGA	TAGTGTTCAC
961	CCTTGTTACA	CCGTTTTTCA	TGAGCAAAC	GAAACGTTTT	CATCGCTCTG	GAGTGAATAC
1021	CACGACGATT	TCCGGCAGTT	TCTACACATA	TATTCGCAAG	ATGTGGCGTG	TTACGGTGAA
1081	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT	GAGAATATGT	TTTTCGTCTC	AGCCAATCCC
1141	TGGGTGAGTT	TCACCAAGTT	TGATTTAAAC	GTGGCCAATA	TGGACAACCT	CTTCGCCCCC
1201	GTTTTACCA	TGGGCAAATA	TTATACGCAA	GGCGACAAGG	TGCTGATGCC	GCTGGCGATT
1261	CAGGTTTCATC	ATGCCGTCTG	TGATGGCTTC	CATGTCGGCA	GAATGCTTAA	TGAATTACAA
1321	CAGTACTGCG	ATGAGTGGCA	GGGCGGGGCG	TAAACGCGTG	GATCCGGCTT	ACTAAAAGCC
1381	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT	TTTTGCGGTA	TAAGAATATA	TACTGATATG
1441	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG	CTATGAAGCA	GCGTATTACA	GTGACAGTTG
1501	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT	ATATGATGTC	AATATCTCCG	GTCTGGTAAG
1561	ACAACCATG	CAGAATGAAG	CCCGTCGTCT	GCGTGCCGAA	CGCTGGAAAG	CGGAAAATCA
1621	GGAAGGGATG	GCTGAGGTG	CCCGGTTTAT	TGAAATGAAC	GGCTCTTTTG	CTGACGAGAA
1681	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT	ACACCTATAA	AAGAGAGAGC	CGTTATCGTC
1741	TGTTTGTGGA	TGTACAGAGT	GATATTATTG	ACACGCCCGG	GCGACGGATG	GTGATCCCCC
1801	TGGCCAGTGC	ACGTCTGCTG	TCAGATAAAG	TCTCCCGTGA	ACTTTACCCG	GTGGTGCATA
1861	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA	CCGATATGGC	CAGTGTGCCG	GTCTCCGTTA
1921	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC	GCGAAAATGA	CATCAAAAAC	GCCATTAAAC
1981	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT	CCCTTATACA	CAGCCAGTCT	GCAGGTCGAC
2041	CATAGTGACT	GGATATGTTG	TGTTTTACAG	TATTATGTAG	TCTGTTTTTT	ATGCAAAATC
2101	TAATTTAATA	TATTGATATT	TATATCATT	TACGTTTCTC	GTTTCAGCTTT	CTTGTACAAA
2161	GTGGTGATGA	TCCGGCTGCT	AACAAAGCCC	GAAAGGAAGC	TGAGTTGGCT	CTGCCACCG
2221	CTGAGCAATA	ACTAGCATAA	CCCCTTGGGG	CCTCTAAACG	GGTCTTGAGG	GGTTTTTTTG
2281	TGAAAGGAGG	AACTATATCC	GGATATCCAC	AGGACGGGTG	TGGTCGCCAT	GATCGCGTAG
2341	TCGATAGTGG	CTCCAAGTAG	CGAAGCGAGC	AGGACTGGGC	GGCGGCCAAA	GCGGTCCGAC
2401	AGTGCTCCGA	GAACGGGTGC	GCATAGAAAT	TGCATCAACG	CATATAGCGC	TAGCAGCAGC
2461	CCATAGTGAC	TGGCGATGCT	GTCCGAATGG	ACGATATCCC	GCAAGAGGCC	CGGCAGTACC
2521	GGCATAACCA	AGCCTATGCC	TACAGCATCC	AGGGTGACGG	TGCCGAGGAT	GACGATGAGC
2581	GCATTGTTAG	ATTTCATACA	CGGTGCCTGA	CTGCGTTAGC	AATTTAACTG	TGATAAACTA
2641	CCGCATTAAA	GCTTATCGAT	GATAAGCTGT	CAAACATGAG	AATTCTTGAA	GACGAAAAGG
2701	CCTCGTGATA	CGCCTATTTT	TATAGGTAA	TGTCATGATA	ATAATGGTTT	CTTAGACGTC
2761	AGGTGGCACT	TTTCGGGGAA	ATGTGCGCGG	AACCCCTATT	TGTTTATTTT	TCTAAATACA-

Figure 36B



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2821 TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA  
2881 AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTGCCCCTT ATTCCCTTTT TTGCGGCATT  
2941 TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA  
3001 GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG  
3061 TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCCTGC TATGTGGCGC  
3121 GGTATTATCC CGTGTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA  
3181 GAATGACTTG GTTGAGTACT CACCACTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT  
3241 AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT  
3301 GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT  
3361 AACTCGCCTT GATCGTTGGG AACCAGGAGT GAATGAAGCC ATACCAAACG ACGAGCGTGA  
3421 CACCACGATG CCTGCAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACCTG GCGAACTACT  
3481 TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAAGGACC  
3541 ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA  
3601 CGGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAAGCCCT CCCGTATCGT  
3661 AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA  
3721 GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT  
3781 TTAGATTGAT TTAAAACTTC ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA  
3841 TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT  
3901 AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA  
3961 AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT  
4021 TTTTCCGAAG GTAACCTGGT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA  
4081 GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACC GCTACATACC TCGCTCTGCT  
4141 AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGACTC  
4201 AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA  
4261 GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA  
4321 AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG  
4381 AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCTCTG  
4441 CGGGTTTTCG CACCTCTGAC TTGAGCGTCT ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG  
4501 CCTATGGAAA AACGCCAGCA ACGCGGCCCT TTTACGGTTC CTGGCCCTTT GCTGGCCCTT  
4561 TGCTCACATG TTCTTTCCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT  
4621 TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA  
4681 GGAAGCGGAA GAGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA  
4741 CCGCATATAT GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT  
4801 ACACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG  
4861 CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG  
4921 TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGGCAGC  
4981 TGCGGTAAAG CTCATCAGCG TGGTCTGTGA GCGATTCA CA GATGTCTGCC TGTTTATCCG  
5041 CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG TTAATGTCTG GCTTCTGATA AAGCGGGCCA  
5101 TGTTAAGGGC GGTTTTTTCC TGTTTGGTCA CTGATGCCTC CGTGTAAGGG GGATTTCTGT  
5161 TCATGGGGGT AATGATACCG ATGAAACGAG AGAGGATGCT CACGATACGG GTTACTGATG  
5221 ATGAACATGC CCGGTTACTG GAACGTTGTG AGGGTAAACA ACTGGCGGTA TGGATGCGGC  
5281 GGGACCAGAG AAAAATCACT CAGGGTCAAT GCCAGCGCTT CGTTAATACA GATGTAGGTG  
5341 TTCCACAGGG TAGCCAGCAG CATCCTGCGA TGCAGATCCG GAACATAATG GTGCAGGGCG  
5401 CTGACTTCCG CGTTTCCAGA CTTTACGAAA CACGGAAACC GAAGACCATT CATGTTGTTG  
5461 CTCAGGTGCG AGACGTTTTG CAGCAGCAGT CGCTTCAGT TCGCTCGCGT ATCGGTGATT  
5521 CATTCTGCTA ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCTCTAAC GACAGGAGCA  
5581 CGATCATGCG CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCGC GTGCGGCTGC  
5641 TGGAGATGGC GGACGCGATG GATATGTTCT GCCAAGGGTT GGTTCGCGA TTCACAGTTC  
5701 TCCGCAAGAA TTGATTGGCT CCAATTCTTG GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC  
5761 GGCTTCCATT CAGGTCGAGG TGGCCCGGCT CCATGCACCG CGACGCAACG CCGGGAGGCA  
5821 GACAAGGTAT AGGGCGGGC CTACAATCCA TGCCAACCCG TTCCATGTGC TCGCCGAGGC  
5881 GGCATAAATC GCCGTGACGA TCAGCGGTCC AGTGATCGAA GTTAGGCTGG TAAGAGCCCG  
5941 GAGCGATCCT TGAAGCTGTG CCTGATGGTC GTCATCTACC TGCCTGGACA GCATGGCCTG  
6001 CAACGCGGGC ATCCCGATGC CGCCGGAAGC GAGAAGAATC ATAATGGGGA AGGCCATCCA  
6061 GCCTCGCGTC GCGAACGCCA GCAAGACGTA GCCCAGCGCG TCGGCCGCCA TGCCGGCGAT  
6121 AATGGCCTGC TTCTCGCCGA AACGTTTGGT GCGGGGACCA GTGACGAAGG CTTGAGCGAG  
6181 GCGTGCAAG ATTCGAATA CCGCAAGCGA CAGGCCGATC ATCGTCTGCG TCCAGCGAAA  
6241 GCGTCTCTCG CCGAAAATGA CCCAGAGCGC TGCCGGCACC TGCTCTACGA GTTGCATGAT

FIGURE 36C

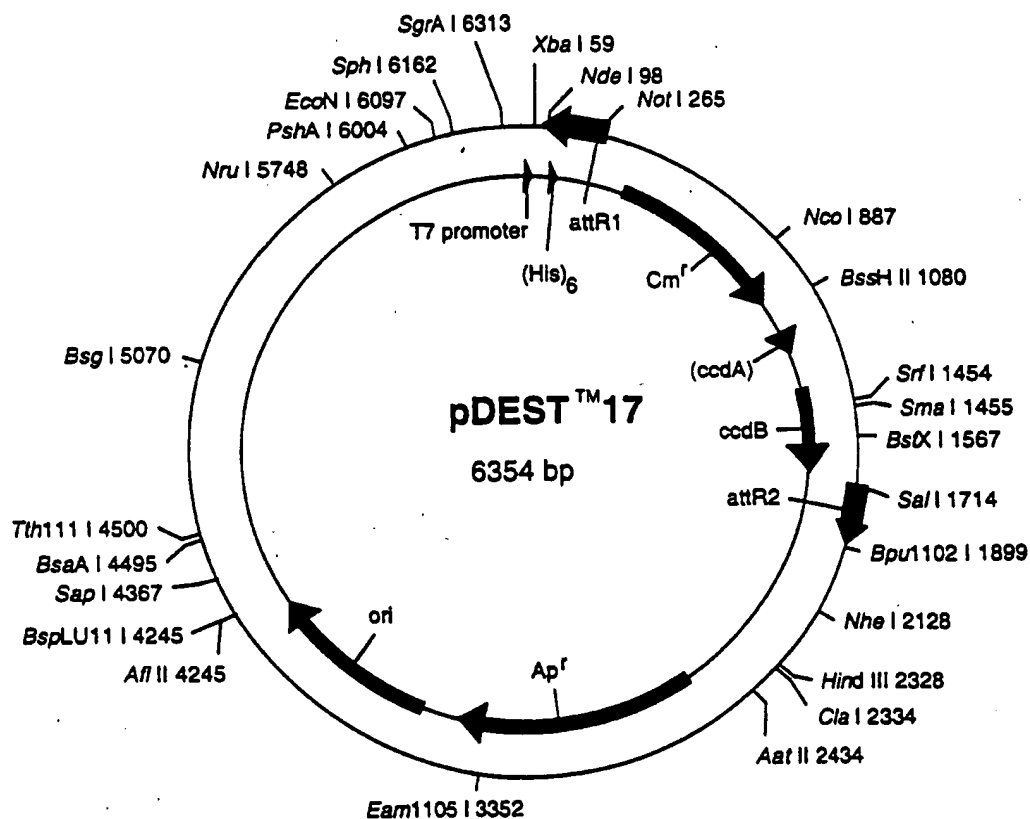
94/240

6301 AAAGAAGACA GTCATAAGTG CGGCGACGAT AGTCATGCCC CGCGCCCACC GGAAGGAGCT  
6361 GACTGGGTTG AAGGCTCTCA AGGGCATCGG TCGATCGACG CTCTCCCTTA TGCGACTCCT  
6421 GCATTAGGAA GCAGCCCAGT AGTAGGTTGA GGCCGTTGAG CACCGCCGCC GCAAGGAATG  
6481 GTGCATGCAA GGAGATGGCG CCCAACAGTC CCCCAGGCCAC GGGGCCTGCC ACCATACCCA  
6541 CGCCGAAACA AGCGCTCATG AGCCCGAAGT GGCGAGCCCG ATCTTCCCA TCGGTGATGT  
6601 CGGCGATATA GCGCCAGCA ACCGCACCTG TGGCGCCGGT GATGCCGGCC ACGATGCGTC  
6661 CGGCGTAGAG GATCG

FIGURE 360

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1 gat ccc gcg aaa tca ata cga ctc act ata ggg aga cca caa cgg ttt ccc  
 cta ggg cgc ttt aat tat gct gag tga tat ccc tct ggt gtt gcc aaa ggg  
 52 tct aga aat aat ttt gtt taa ctt taa gaa gga gat ata cat atg tgg tac  
 aga tct tta tta aaa caa att gaa att ctt cct cta tat gta tac agc atg  
 103 Y H H H H H H L E S T S L Y K K A aca agt tgg tac aaa aaa gct  
 tac cat cac cat cac cat cac ctc gaa tca tgt tca aac atg ttt ttt cga  
 atg gta gtg gta gtg gta gtg gag ctt agt attR1 Int



## pDEST17 6354 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
258..134		attR1
367..1026		CmR
1146..1230		inactivated ccdA
1368..1673		ccdB
1714..1838		attR2
2564..3421		ampR
1	CGATCCCGCG AAATTAATAC GACTCACTAT AGGGGAGACCA CAACGGTTTC CCTCTAGAAA	
61	TAATTTTGTT TAACTTTAAG AAGGAGATAT ACATATGTCTG TACTACCATC ACCATCACCA	
121	TCACCTCGAA TCAACAAGTT TGTACAAAAA AGCTGAACGA GAAACGTAAA ATGATATAAA	
181	TATCAATATA TTAAATTAGA TTTTGCATAA AAAACAGACT ACATAATACT GTAAAACACA	
241	ACATATCCAG TCACATATGGC GGCCGCATTA GGCACCCAG GCTTTACACT TTATGCTTCC	
301	GGCTCGTATA ATGTGTGGAT TTTGAGTTAG GATCCGTCGA GATTTTCAGG AGCTAAGGAA	
361	GCTAAAATGG AGAAAAAAT CACTGGATAT ACCACCGTTG ATATATCCCA ATGGCATCGT	
421	AAAGAACATT TTGAGGCATT TCAGTCAGTT GCTCAATGTA CCTATAACCA GACCGTTCAG	
481	CTGGATATTA CGGCCTTTTT AAAGACCGTA AAGAAAAATA AGCACAAGTT TTATCCGGCC	
541	TTTATTACACA TTCTTGCCCG CCTGATGAAT GCTCATCCGG AATTCCGTAT GGCAATGAAA	
601	GACGGTGAGC TGGTGATATG GGATAGTGTT CACCCTTGTT ACACCGTTTT CCATGAGCAA	
661	ACTGAAACGT TTTTCATCGCT CTGGAGTGAA TACCACGACG ATTTCCGGCA GTTTCACAC	
721	ATATATTTCG AAGATGTGGC GTGTTACGGT GAAAACCTGG CCTATTTCCC TAAAGGGTTT	
781	ATTGAGAATA TGTTTTTCGT CTCAGCCAAT CCCTGGGTGA GTTTCACCAG TTTTGATTTA	
841	AACGTGGCCA ATATGGACAA CTTCCTCGCC CCCGTTTTCA CCATGGGCAA ATATTATACG	
901	CAAGGCGACA AGGTGCTGAT GCCGCTGGCG ATTCAGGTC ATCATGCCGT CTGTGATGGC	
961	TTCCATGTCG GCAGAATGCT TAATGAATTA CAACAGTACT GCGATGAGTG GCAGGGCGGG	
1021	GCGTAAAGAT CTGGATCCGG CTACTAAAA GCCAGATAAC AGTATGCGTA TTTGCGCGCT	
1081	GATTTTTGCG GTATAAGAAT ATATACTGAT ATGTATACCC GAAGTATGTC AAAAAGAGGT	
1141	GTGCTATGAA GCAGCGTATT ACAGTGACAG TTGACAGCGA CAGCTATCAG TTGCTCAAGG	
1201	ATTATATGAT GTCAATATCT CCGGTCTGGT AAGCACAACC ATGCAGAATG AAGCCCGTCG	
1261	TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG ATGGCTGAGG TCGCCCGGTT	
1321	TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC TGGTGAAATG CAGTTTAAAGG	
1381	TTTACACCTA TAAAAGAGAG AGCCGTTATC GTCTGTTTTGT GGATGTACAG AGTGATATTA	
1441	TTGACACGCC CGGGCGACGG ATGGTGATCC CCCTGGCCAG TGCACGTCGT CTGTCAGATA	
1501	AAGTCTCCCG TGAACTTTAC CCGGTGGTGC ATATCGGGGA TGAAAGCTGG CGCATGATGA	
1561	CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA AGAAGTGGCT GATCTCAGCC	
1621	ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT CTGGGGAATA TAAATGTCAG	
1681	GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG ACTGGATATG TTGTGTTTTA	
1741	CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA ATATATTGAT ATTTATATCA	
1801	TTTACGTTTT CTCGTTACGC TTTCTTGAC AAAGTGGTTG ATTCGAGGCT GCTAACAAAG	
1861	CCCGAAAGGA AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA ATAAGTAGCA TAACCCCTTG	
1921	GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGCTGAAAGG AGGAAGTATA TCCGGATATC	
1981	CACAGGACGG GTGTGGTCGC CATGATCGCG TAGTCGATAG TGGCTCCAAG TAGCGAAGCG	
2041	AGCAGGACTG GGCGGCGGCC AAAGCGGTCTG GACAGTGCTC CGAGAACGGG TGCGCATAGA	
2101	AATTGCATCA ACGCATATAG CGCTAGCAGC ACGCCATAGT GACTGGCGAT GCTGTGCGAA	
2161	TGGACGATAT CCCGCAAGAG GCGCGGCAGT ACCGGCATAA CCAAGCCTAT GCCTACAGCA	
2221	TCCAGGGTGA CCGTGCCGAG GATGACGATG AGCGCATTGT TAGATTTTCAT ACACGGTGCC	
2281	TGACTGCGTT AGCAATTTAA CTGTGATAAA CTACCGCATT AAAGCTTATC GATGATAAGC	
2341	TGTCAAACAT GAGAATTTCT GAAGACGAAA GGGCCTCGTG ATACGCCTAT TTTTATAGGT	
2401	TAATGTCATG ATAATAATGG TTTCTTAGAC GTCAGGTGGC ACTTTTCGGG GAAATGTGCG	
2461	CGGAACCCCT ATTTGTTTAT TTTTCTAAAT ACATTCAAAT ATGTATCCGC TCATGAGACA	
2521	ATAACCCCTGA TAAATGCTTC AATAATATTG AAAAAGGAAG AGTATGAGTA TTCAACATTT	
2581	CCGTGTCGCC CTTATTCCCT TTTTTCGGGC ATTTTGCCCTT CCTGTTTTTG CTCACCCAGA	
2641	AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT GCACGAGTGG GTTACATCGA-	

FIGURE 37B

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2701 ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTTCGC CCCGAAGAAC GTTTTCCAAT  
 2761 GATGAGCACT TTTAAAGTTC TGCTATGTGG CGCGGTATTA TCCCGTGTG ACGCCGGGCA  
 2821 AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT  
 2881 CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC  
 2941 CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC CGAAGGAGCT  
 3001 AACCCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAAACGGGA  
 3061 GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGCAG CAATGGCAAC  
 3121 AACGTTGCGC AAACCTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAAATTAAT  
 3181 AGACTGGATG GAGGCGGATA AAGTTGCAAG ACCACTTCTG CGCTCGGCC TTCCGGCTGG  
 3241 CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG TCTCGCGGTA TCATTGCAGC  
 3301 ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG GGAGTCAGGC  
 3361 AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG  
 3421 GTAACGTGCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAC TTCATTTTTTA  
 3481 ATTTAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA TCCCTTAACG  
 3541 TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA  
 3601 TCCTTTTTCG CTGCGCTGTA TCTGCTGCTT GCAAACAAA AAACCACCGC TACCAGCGT  
 3661 GGTTCGTTTG CCGGATCAAG AGCTACCAAC TCTTTTCCG AAGGTAACGT GCTTCAGCAG  
 3721 AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC ACTTCAAGAA  
 3781 CTCTGTAGCA CCGCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG  
 3841 TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA  
 3901 GCGGTCCGGC TGAACGGGGG GTTCGTGCAC ACAGCCCGC TTGGAGCGAA CGACCTACAC  
 3961 CGAACTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA  
 4021 GGCGGACAGG TATCCGGTAA GCGGCAGGCT CGGAACAGGA GAGCGCACGA GGGAGCTTCC  
 4081 AGGGGGAAC GCCTCGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT GACTTGAGCG  
 4141 TCGATTTTTC TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCGGC  
 4201 CTTTTTACGG TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC ATGTTCTTTC CTGCGTTATC  
 4261 CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGCGAG  
 4321 CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC TGATGCGGTA  
 4381 TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA TATGGTGCAC TCTCAGTACA  
 4441 ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACACTCC GCTATCGCTA CGTGATGGG  
 4501 TCATGCTGCG GCCCCGACAC CCGCTGACGC GCCCTGACGG GCTTGTCTGG  
 4561 TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT  
 4621 TTTACCGTTC ATCACCGAAA CGCGCGAGGC AGCTGCGGTA AAGCTCATCA GCGTGGTCTG  
 4681 GAAGCGATTTC ACAGATGTCT GCCTGTTTCAT CCGCGTCCAG CTCGTTGAGT TTCTCCAGAA  
 4741 GCGTTAATGT CTGGCTTCTG ATAAAGCGGG CCATGTTAAG GCGGGTTTTT TCCTGTTTGG  
 4801 TCACTGATGC CTCCGTGTAA GGGGGATTTC TGTTTCATGGG GGTAAATGATA CCGATGAAAC  
 4861 GAGAGAGGAT GCTCACGATA CGGGTACTTG ATGATGAACA TGCCCGGTTA CTGGAACGTT  
 4921 TTGAGGGTAA ACAACTGGCG GTATGGATGC GCGGGGACCA GAGAAAAATC ACTCAGGCTC  
 4981 AATGCGAGCG CTTCTGTTAAT ACAGATGTAG GTGTTCCACA GGGTAGCCAG CAGCATCCTG  
 5041 CGATGCAGAT CCGGAACATA ATGGTGCAGG GCGCTGACTT CCGCGTTTCC AGACTTTTACG  
 5101 AAACACGGAA ACCGAAGACC ATTTCATGTTG TTGCTCAGGT CGCAGACGTT TTGCAGCAGC  
 5161 AGTCGCTTCA CGTTCGCTCG CGTATCGGTG ATTCATTCTG CTAACCACTA AGGCAACCCC  
 5221 GCCAGCCTAG CCGGTCTCTC AACGACAGGA GCACGATCAT GCGCACCCGT GGCCAGGACC  
 5281 CAACGCTGCC CGAGATGCGC CGCGTGCGGC TGCTGGAGAT GGCGGACGCG ATGGATATGT  
 5341 TCTGCCAAGG GTTGGTTTGC GCATTACAGC TTCTCCGCAA GAATTGATTG GCTCCAATTC  
 5401 TTGGAGTGTT GAATCCGTTA GCGAGGTGCC GCCGGCTTCC ATTCAGGTG AGGTGGCCCG  
 5461 GCTCCATGCA CCGCGACGCA ACGCGGGGAG GCAGACAAGG TATAGGGCGG CGCCTACAAT  
 5521 CCATGCCAAC CCGTTCCATG TGCTCGCCGA GGCGGCATAA ATCGCCGTGA CGATCAGCGG  
 5581 TCCAGTGATC GAAGTTAGGC TGGTAAGAGC CGCGAGCGAT CCTTGAAGCT GTCCCTGATG  
 5641 GTCGTCATCT ACCTGCCTGG ACAGCATGGC CTGCAACGCG GGCATCCCGA TGCCGCCGGA  
 5701 AGCGAGAAGA ATCATAATGG GGAAGGCCAT CCAGCCTCGC GTCGCGAAGC CCAGCAAGAC  
 5761 GTAGCCGAGC GCGTCGGCCG CCATGCCGGC GATAATGGCC TGCTTCTCGC CGAAACGTTT  
 5821 GGTGGCGGGA CCAGTGACGA AGGCTTGAGC GAGGGCGTGC AAGATTCCGA ATACCGCAAG  
 5881 CGACAGGCCG ATCATCGTCG CGCTCCAGCG AAAGCGGTCC TCGCCGAAAA TGACCCAGAG  
 5941 CGCTGCCGGC ACCTGTCTTA CGAGTTGCAT GATAAAGAAG ACAGTCATAA GTGCGGCGAC  
 6001 GATAGTCATG CCCCAGCCCC ACCGGAAGGA GCTGACTGGG TTGAAGGCTC TCAAGGGCAT  
 6061 CCGTCGATCG ACGCTCTCCC TTATGCGACT CCTGCATTAG GAAGCAGCCC AGTAGTAGGT  
 6121 TGAGGCCGTT GAGCACCGCC GCCGCAAGGA ATGGTGCATG CAAGGAGATG GCGCCCAACA-

FIGURE 37C

6181 GTCCCCCGGC CACGGGGCCT GCCACCATAC CCACGCCGAA ACAAGCGCTC ATGAGCCCGA  
6241 AGTGGCGAGC CCGATCTTCC CCATCGGTGA TGTCGGCGAT ATAGGCGCCA GCAACCGCAC  
6301 CTGTGGCGCC GGTGATGCCG GCCACGATGC GTCCGGCGTA GAGGATCGAG ATCT

FIGURE 37D

Figure 38A: pDEST18

FastBac Transfer Vector with p10  
Baculovirus Promoter

1 gaagacctcg gccgtcgccg cgtttgccgg tgggtgctgac cccggatgaa gtggttcgca  
cttctggagc cggcagcgcc gcgaacggcc accacgactg gggcctactt caccaagcgt

61 tcttcgggttt tetggaagge gagcatcggtt tggtcgccca ggactctagc tatagttcta  
aggagccaaa agaccttccg ctctagtagcaa acaagcgggt cctgagatcg atatcaagat

121 gtggtttggct acgtatcgag caagaaccta aaagccaaa cgcgttggag tcttctgttc  
caccaaccga tgcatagtct gttcttttat ttcgggttt gcgaacctc agaacacacg

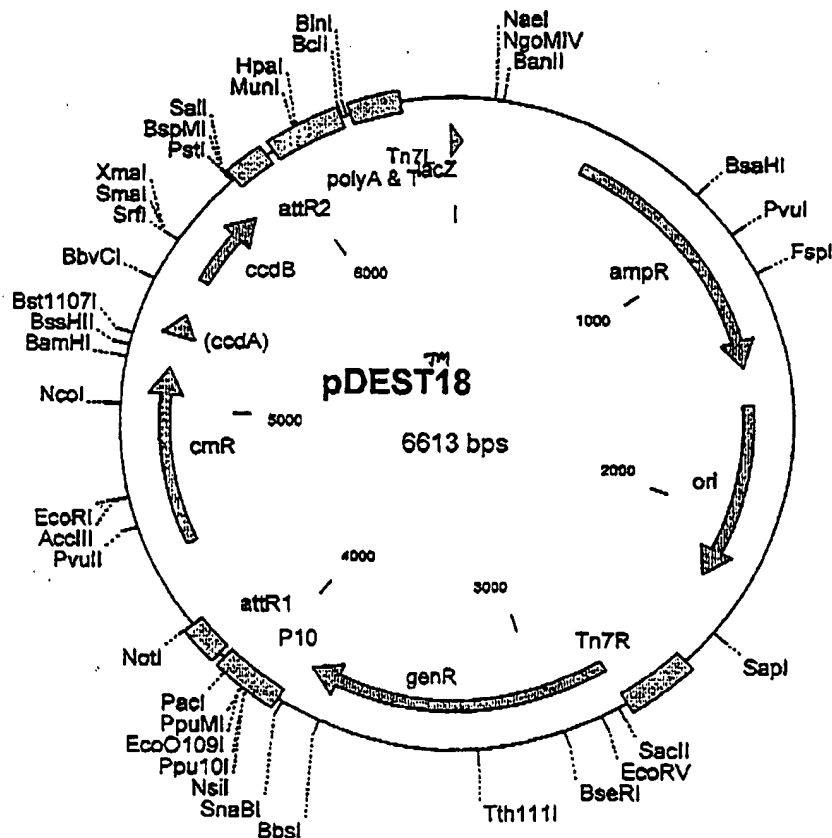
181 11 tatctttaca agatctcaga atactgcata accacacaa cgggggacta caaatatag 11  
11 aaagaaatgt ttctaaatct ttatgcctag tgaatgttgt tccccctgat acittaatat 11

241 11 caatttcgag gtgcggggac ctttaattca acccaacaca atatattata gtaaatatag 11  
11 gtaaaactcc tacggccctg gaaattgaat tgggttgtat tatataatat caatttatc 11

301 11 atttatatat caaatcattt gtatattaat taataatact tactgttaat tacattttat 11  
11 taataaata gtttagtaaa catataatta attttatgat atgacattta acgtaanata 11

361 ttacaatgag gatcatcaca agtttgtaca aaaaaagctga acgagaaaacg taaaatgata  
aatgttactc ctagtagtgt tcaaacatgt ttttcgact tgctctttgc attttactat

Int. attR1



## pDEST18 6613 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
474..1449		ampR
1590..2244		ori
2738..3850		genR
4251..4127		attR1
4501..5160		CmR
5280..5364		inactivated ccdA
5502..5807		ccdB
5848..5972		attR2
6595..25		lacZ
1	GACGCGCCCT GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC	
61	GCTACACTTG CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC	
121	ACGTTTCGCG GCTTTCCCGG TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT	
181	AGTGCTTTAC GGCACCTCGA CCCCCAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG	
241	CCATCGCCCT GATAGACGGT TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT	
301	GGACTCTTGT TCCAAACTGG AACAACACTC AACCCATATCT CGGTCTATTC TTTTGATTTA	
361	TAAGGGATTT TGCCGATTTC GGCCTATTGG TTAAAAAATG AGCTGATTTA ACAAAAATTT	
421	AACGCGAATT TTAACAAAAT ATTAACGTTT ACAATTTTTC GTGGCACTTT TCGGGGAAAT	
481	GTGCGCGGAA CCCCTATTTC TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG	
541	AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA	
601	CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC	
661	CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC	
721	ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT	
781	CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC	
841	GGGCAAGAGC AACTCGGTCG CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA	
901	CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC	
961	ATAACCATGA GTGATAACAC TCGGCCCAAC TTAATTCTGA CAACGATCGG AGGACCGAAG	
1021	GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCCTGA TCGTTGGGAA	
1081	CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG	
1141	GCAACAACGT TCGCCAAACCT ATTAAGTGGC GAAGTACTTA CTCTAGCTTC CCGGCAACAA	
1201	TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG	
1261	GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT	
1321	GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT	
1381	CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG	
1441	CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT	
1501	TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT	
1561	TAACGTGAGT TTTTCGTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT	
1621	TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA	
1681	GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC	
1741	AGCAGAGCGC AGATACCAA TACTGTCCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC	
1801	AAGAACTCTG TAGCACCACC TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT	
1861	GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG	
1921	GCGCAGCGGT CGGGCTGAAC GGGGGGTTTCG TGCACACAGC CCAGCTTGA GCGAACGACC	
1981	TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG	
2041	AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG	
2101	CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTCT GGTTCGCGCA CCTCTGACTT	
2161	GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGAAAAA CGCCAGCAAC	
2221	GCGGCCCTTT TACGGTTCCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCTGCG	
2281	TTATCCCTG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC	
2341	CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCTGATG	
2401	CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACCC GCAGACCAGC CGCGTAACCT	
2461	GGCAAAATCG GTTACGGTTG AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGCGGGA-	

FIGURE 38B



2521 CAATAAAGTC TTAAACTGAA CAAAATAGAT CTAAACTATG ACAATAAAGT CTTAAACTAG  
2581 ACAGAAATAGT TGTAAACTGA AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGAATTT  
2641 TGTTATGGCT AAAGCAAACCT CTTCAATTTTC TGAAGTGCAA ATTGCCCGTC GTATTAAAGA  
2701 GGGGCGTGGC CAAGGGCATG GTAAAGACTA TATTCGCGGC GTTGTGACAA TTTACCSAAC  
2761 AACTCCGCGG CCGGGAGGCC GATCTCGGCT TGAACGAATT GTTAGGTGGC GTACTTGGG  
2821 TCGATATCAA AGTGCATCAC TTCTTCCCGT ATGCCCAACT TTGTATAGAG AGCCACTGCG  
2881 GGATCGTCAC CGTAATCTGC TTGCACGTAG ATCACATAAG CACCAAGCGC GTTGGCTCA  
2941 TGCTTGAGGA GATTGATGAG CGCGGTGGCA ATGCCCTGCC TCCGGTGTCT CCGGAGACT  
3001 GCGAGATCAT AGATATAGAT CTCACTACGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC  
3061 GCGAGAGCGC CAACAACCGC TTCTTGGTCG AAGGCAGCAA GCGCGATGAA TGTCTTACTA  
3121 CGGAGCAAGT TCCCGAGGTA ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT  
3181 CCGAACTCAC GACCGAAAAG ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGSCCG  
3241 AGCCTACATG TCGAATGAT GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG  
3301 CCCTGCTGCG TAACATCGTT GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAACA  
3361 TCGACCCACG GCGTAACGCG CTTGTGCTT GGATGCCCGA GGCATAGACT GTACAAAAA  
3421 ACAGTCATAA CAAGCCATGA AAACCGCCAC TCGCGCGTTA CCACCGCTGC GTTCGGTCAA  
3481 GGTCTCTGGAC CAGTTGCGTG AGCGCATACG CTACTTGCAT TACAGTTTAC GAACCGAACA  
3541 GGCTTATGTC AACTGGGTTC GTGCCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC  
3601 CTTGGGCAGC AGCGAAGTCG AGGCATTTCT GTCTTGGCTG GCGAACGAGC GCAAGGTTTC  
3661 GGTCTCCACG CATCGTCAGG CATTGCGGCG CTTGCTGTTT TTCTACGGCA AGGTGCTGTG  
3721 CACGGATCTG CCCTGGCTTC AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGCCTGT  
3781 GGTGCTGACC CCGGATGAAG TGGTTCGCAT CCTCGGTTTT CTGGAAGGCG AGCATCTTTT  
3841 GTTCGCCCCG GACTCTAGCT ATAGTTCTAG TGGTGGCTA CGTATCGAGC AAGAAATATA  
3901 AACGCCAAAC GCGTTGGAGT TTGTGTGCT ATTTTACAA AGATTGAGAA ATACGCATCA  
3961 CTTACAACAA GGGGGACTAT GAAATTATGC ATTTTGAGGA TGCCGGGACC TTTAATTCAA  
4021 CCCAACACAA TATATTATAG TTAAATAAGA ATTATTTATC AAATCATTTG TATATTAAAT  
4081 AAAATACTAT ACTGTAAATT ACATTTTATT TACAATGAGG ATCATCACAA GTTTGTACAA  
4141 AAAAGCTGAA CGAGAAACGT AAAATGATAT AAATATCAAT ATATTAATTT AGATTTTGCA  
4201 TAAAAAACAG ACTACATAAT ACTGTAAATC ACAACATATC CAGTCACTAT GCGGCGCGCT  
4261 AAGTTGGCAG CATCACCCGA CGCACTTTGC GCCGAATAAA TACCTGTGAC GGAAGATCAC  
4321 TTGCGAGAAT AAATAAATCC TGGTGTCCCT GTTGATACCG GGAAGCCCTG GGCCAACTTT  
4381 TGGCGAAAAT GAGACGTTGA TCGGCACGTA AGAGGTTCCA ACTTTCACCA TAATGAAATA  
4441 AGATCACTAC CGGGCGTATT TTTTGAGTTA TCGAGATTTT CAGGAGCTAA GGAAGCTAAA  
4501 ATGGAGAAAA AAATCACTGG ATATACCACC GTTGATATAT CCCAATGGCA TCGTAAAGAA  
4561 CATTTTGAGG CATTTCAGTC AGTTGCTCAA TGTACCTATA ACCAGACCGT TCAGCTGAT  
4621 ATTACGGCCT TTTTAAAGAC CGTAAAGAAA AATAAGCACA AGTTTATCC GGCCTTTATT  
4681 CACATTCTTG CCCGCTGAT GAATGCTCAT CCGGAATTCC GTATGGCAAT GAAAGACGGT  
4741 GAGCTGGTGA TATGGGATAG TGTTACCCCT TGTTACACCG TTTTCCATGA GCAAACGTAA  
4801 ACGTTTTTCAT CGCTCTGGAG TGAATACCAC GACGATTTCC GGCAGTTTCT ACACATATAT  
4861 TCGCAAGATG TGGCGTGTGA CGGTGAAAAC CTGGCCTATT TCCCTAAAGG GTTTATTGAG  
4921 AATATGTTTT TCGTCTCAGC CAATCCCTGG GTGAGTTTCA CCAGTTTGA TTTAAACGTG  
4981 GCCAATATGG ACAACTTCTT CGCCCCCGTT TTCACCATGG GCAAATATTA TACGCAAGGC  
5041 GACAAGGTGC TGATGCCGCT GCGGATTCAG GTTCATCATG CCGTCTGTGA TGGCTTCCAT  
5101 GTCGGCAGAA TGCTTAATGA ATTACAACAG TACTGCGATG AGTGGCAGGG CCGGGCSTAA  
5161 ACGCGTGGAT CCGGCTTACT AAAAGCCAGA TAACAGTATG CGTATTGCG CGCTGATTTT  
5221 TGCGGTATAA GAATATATAC TGATATGTAT ACCCGAAGTA TGTCAAAAAG AGGTGTCTA  
5281 TGAAGCAGCG TATTACAGTG ACAGTTGACA GCGACAGCTA TCAGTTGCTC AAGGCATATA  
5341 TGATGTCAAT ATCTCCGCTC TGGTAAGCAC AACCATGCAG AATGAAGCCC GTCGTCTGCG  
5401 TGCCGAACGC TGGAAAGCGG AAAATCAGGA AGGGATGGCT GAGGTCGCCC GGTTTATGTA  
5461 AATGAACGGC TCTTTTGCTG ACGAGAACAG GGAAGTGTGA AATGCAGTTT AAGGTTTACA  
5521 CCTATAAAAG AGAGAGCCGT TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA  
5581 CGCCCCGGCG ACGGATGGTG ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAACTCT  
5641 CCCGTGAAC TTAACCGGTG GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG  
5701 ATATGGCCAG TGTGCCGCTC TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG  
5761 AAAATGACAT CAAAAACGCC ATTAACCTGA TGTTCTGGGG AATATAAATG TCAGGCTCCC  
5821 TTATACACAG CCAGTCTGCA GGTGACCAT AGTGACTGGA TATGTTGTGT TTTACAGTAT  
5881 TATGTAGTCT GTTTTTTATG CAAAATCTAA TTAAATATAT TGATATTTAT ATCATTTTAC  
5941 GTTCTCGTT CAGCTTCTT GTACAAAGTG GTGATAGCTT GTCGAGAAGT ACTAGAGGAT-

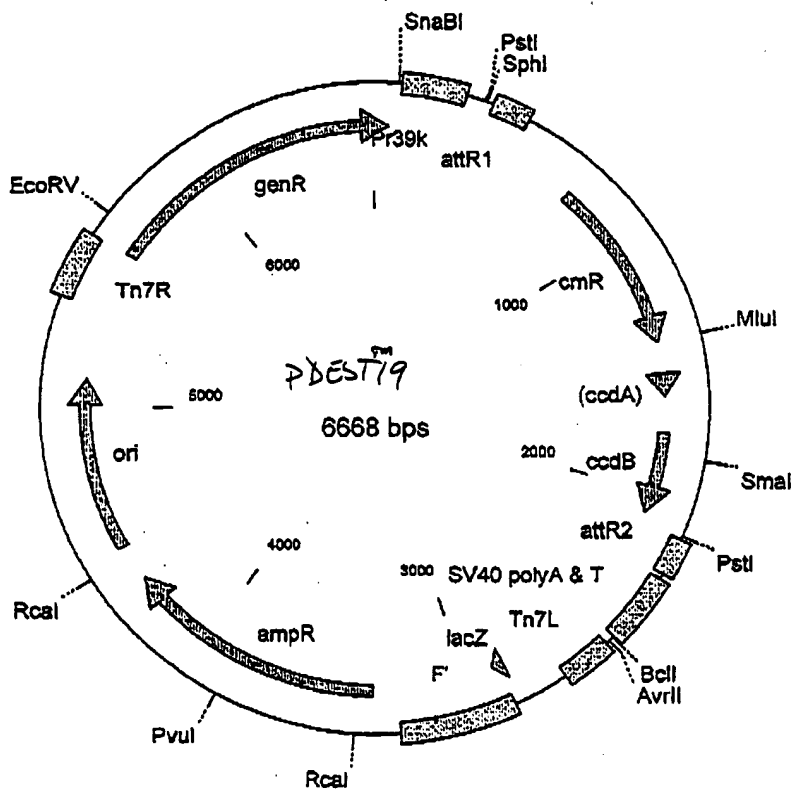
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6001 CATAATCAGC CATACCACAT TTGTAGAGGT TTTACTTGCT TTAAAAAACC TCCCACACCT  
6061 CCCCCTGAAC CTGAAACATA AAATGAATGC AATTGTTGTT GTTAACTTGT TTATTGCAGC  
6121 TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTTT CAAATAAAG CATTTTTTTC  
6181 ACTGCATTCT AGTTGTGGTT TGTCCAACT CATCAATGTA TCTTATCATG TCTGGATCTG  
6241 ATCACTGCTT GAGCCTAGGA GATCCGAACC AGATAAGTGA AATCTAGTTC CAAACTATTT  
6301 TGTCATTTTT AATTTTCGTA TTAGCTTACG ACGCTACACC CAGTCCCAT CTATTTTGTC  
6361 ACTCTTCCCT AAATAATCCT TAAAACTCC ATTTCCACCC CTCCCAGTTC CCAACTATTT  
6421 TGTCCGCCCA CAGCGGGGCA TTTTCTTCC TGTTATGTTT TTAATCAAAC ATCCTGCCAA  
6481 CTCCATGTGA CAAACCGTCA TCTTCGGCTA CTTTTTCTCT GTCACAGAAT GAAAATTTTT  
6541 CTGTCATCTC TTCGTTATTA ATGTTTGTA TTAGTGAAT ATCAACGCTT ATTTGCAGCC  
6601 TGAATGGCGA ATG

FIGURE 38D

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1 ggtgacgccc tcatttttcc attgtaacgt aaatggcaac ttgtagatga acgcgctgtc  
 ccactgcggc agtagaaaagg taacattgca ttaccggtg aacatctact tgcgcgacag  
 61 aaaaaaccgg ccagttttctt ccacaaatc gcgcacggct gtctcgtaaa cttttgcgtc  
 ttttttggcc ggtcaaagaa ggtgtttgag cgcgtgccga cagagcattt gaaaacgcag  
 121 // gcaacaatcg cgatgacctc gtggtatgga aattttttct aaaaaagtgt cgttcattgtc //  
 // cgttggttagc gctactggag caccatacct ttaaaaaaga ttttttcaca gcaagtacag //  
 181 // ggccggggcg ttcgcgctcc ggtacgcgcg acgggcacac agcaggacag cttgtccgg  
 // ccgcgcgcgc aagcgcgagg ccatgcgcgc tgcccggtg tcgtctctgc ggaacaggcc  
 241 ctcgattatc ataaacaatc ctgcaggcat gcaagctgga tcatacaag ttgtacaaa  
 gagctaatag tatttgttag gacgtccgta cgttcgacct agtagtcttc aaacatgctt  
 Int V



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## pDEST19 6668 bp (rotated to position 1000)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
515..391		attR1
765..1424		CmR
1544..1628		inactivated ccdA
1766..2071		ccdB
2112..2236		attR2
2852..2895		lacZ
3344..4319		ampR
4460..5114		ori
5608..52		genR

1	AGTGGTTCGC	ATCCTCGGTT	TTCTGGAAGG	CGAGCATCGT	TTGTTGCCCC	AGGACTCTAG
61	CTATAGTTCT	AGTGGTTGGC	TACGTATATC	AAATACTTGT	AGGTGACGCC	GTCATCTTTC
121	CATTGTAACG	TAAATGGCAA	CTTGATAGATG	AACGCGCTGT	CAAAAAACCG	GCCAGTTTCT
181	TCCACAAACT	CGCGCACGGC	TGTCTCGTAA	ACTTTTGCGT	CGCAACAATC	GCGATGACCT
241	CGTGGTATGG	AAATTTTTC	TAAAAAAGTG	TCGTTTCATGT	CGGCGGCGGG	CGCGTTCGCG
301	CTCCGGTACG	CGCGACGGGC	ACACAGCAGG	ACAGCCTTGT	CCGGCTCGAT	TATCATAAAC
361	AATCCTGCAG	GCATGCAAGC	TCGATCATC	ACAAGTTTGT	ACAAAAAAGC	TGAACGAGAA
421	ACGTAAAATG	ATATAAATAT	CAATATATTA	AATTAGATT	TGCATAAAAA	ACAGACTACA
481	TAATACTGTA	AAACACAACA	TATCCAGTCA	CTATGGCGGC	CGCTAAGTTG	GCAGCATCAC
541	CCGACGCACT	TTGCGCCGAA	TAAATACCTG	TGACGGAAGA	TCACTTCGCA	GAATAAATAA
601	ATCCTGGTGT	CCCTGTTGAT	ACCGGGAAGC	CCTGGGCCAA	CTTTTGCGCA	AAATGAGACG
661	TTGATCGGCA	CGTAAGAGGT	TCCAACTTTC	ACCATAATGA	AATAAGATCA	CTACCGGGCG
721	TATTTTTTGA	GTTATCGAGA	TTTTCAGGAG	CTAAGGAAGC	TAAAATGGAG	AAAAAATCA
781	CTGGATATAC	CACCGTTGAT	ATATCCCAAT	GGCATCGTAA	AGAACATTTT	GAGGCATTTT
841	AGTCAGTTGC	TCAATGTACC	TATAACCAGA	CCGTTTCAGT	GGATATTACG	GCCTTTTAA
901	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT	ATCCGGCCTT	TATTCACATT	CTTGCCCGCC
961	TGATGAATGC	TCATCCGGAA	TTCCGTATGG	CAATGAAAGA	CGGTGAGCTG	GTGATATGGG
1021	ATAGTGGTTC	CCCTTGTTAC	ACCGTTTTTC	ATGAGCAAAC	TGAAACGTTT	TCATCGCTCT
1081	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT	TTCTACACAT	ATATTGCAA	GATGTGGCGT
1141	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA	AAGGGTTTAT	TGAGAATATG	TTTTTCGTCT
1201	CAGCCAATCC	CTGGGTGAGT	TTCAACAGTT	TTGATTTAAA	CGTGCCCAAT	ATGGACAAC
1261	TCTTCGCCCC	CGTTTTTACC	ATGGGCAAAT	ATTATACGCA	AGGCGACAAG	GTGCTGATGC
1321	CGCTGGCGAT	TCAGGTTTAT	CATGCCGTCT	GTGATGGCTT	CCATGTCGGC	AGAATGCTTA
1381	ATGAATTACA	ACAGTACTGC	GATGAGTGGC	AGGGCGGGGC	GTAACGCGT	GGATCCGGCT
1441	TACTAAAAGC	CAGATAACAG	TATGCGTATT	TGCGCGCTGA	TTTTTGCGGT	ATAAGAATAT
1501	ATACTGATAT	GTATACCCGA	AGTATGTCAA	AAAGAGGTGT	GCTATGAAGC	AGCGTATTAC
1561	AGTGACAGTT	GACAGCGACA	GCTATCAGTT	GCTCAAGGCA	TATATGATGT	CAATATCTCC
1621	GGTCTGGTAA	GCACAACCAT	GCAGAAATGAA	GCCCGTCGTC	TGCGTGCCGA	ACGCTGGAAA
1681	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC	GCCCGGTTTA	TTGAAATGAA	CGGCTCTTTT
1741	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA	GTTTAAGGTT	TACACCTATA	AAAGAGAGAG
1801	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG	TGATATTATT	GACACGCCCC	GGCGACGGAT
1861	GGTGATCCCC	CTGGCCAGTG	CACGTCGTCT	GTCAGATAAA	GTCTCCCGTG	AACCTTACCC
1921	GGTGGTGCAT	ATCGGGGATG	AAAGCTGGCG	CATGATGACC	ACCGATATGG	CCAGTGTGCC
1981	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA	TCTCAGCCAC	CGCGAAAATG	ACATCAAAAA
2041	CGCCATTAA	CTGATGTTCT	GGGGAATATA	AATGTCAGGC	TCCCTTATAC	ACAGCCAGTC
2101	TGCAGGTCGA	CCATAGTGAC	TGGATATGTT	GTGTTTACA	GTATTATGTA	GTCTGTTTTT
2161	TATGCAAAAT	CTAATTTAAT	ATATTGATAT	TTATATCATT	TTACGTTTCT	CGTTCAGCTT
2221	TCTTGTACAA	AGTGGTGATC	GAGAAGTACT	AGAGGATCAT	AATCAGCCAT	ACCACATTTG
2281	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA
2341	TGAATGCAAT	TGTTGTTGTT	AACTTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA
2401	ATAGCATCAC	AAATTTTACA	AATAAAGCAT	TTTTTTCAT	GCATTCTAGT	TGTGGTTTGT
2461	CCAACTCAT	CAATGTATCT	TATCATGTCT	GGATCTGATC	ACTGCTTGAG	CCTAGGAGAT
2521	CCGAACCAGA	TAAGTGAAAT	CTAGTTCCAA	ACTATTTTGT	CAITTTTAAT	TTTCGTATTA
2581	GCTTACGACG	CTACACCCAG	TTCCCATCTA	TTTTGTCACT	CTTCCCTAAA	TAATCCTTAA

FIGURE 39B

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2641 AAACCTCCATT TCCACCCCTC CCAGTTCCCA ACTATTTTGT CCGCCACAG CGGGGCATTT  
2701 TTCTTCCTGT TATGTTTTTA ATCAAACATC CTGCCAACTC CATGTGACAA ACCGTCATCT  
2761 TCGGCTACTT TTTCTCTGTC ACAGAATGAA AATTTTTCTG TCATCTCTTC GTTATTAATG  
2821 TTTGTAATTG ACTGAATATC AACGCTTATT TGCAGCCTGA ATGGCGAATG GACGCGCCCT  
2881 GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG  
2941 CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC ACGTTCGCGG  
3001 GCTTTCCTCCG TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT AGTGCTTTAC  
3061 GGCACCTCGA CCCCCAAAAA CTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT  
3121 GATAGACGGT TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT  
3181 TCCAAACTGG AACAACTC AACCCCTATCT CGGTCTATTC TTTTGATTTA TAAGGGATTT  
3241 TGCCGATTTC GGCCTATTGG TTAATAAATG AGCTGATTTA ACAAATAATTT AACCGGAATT  
3301 TTAACAAAAT ATTAACGTTT ACAATTTTCTG GTGGCACTTT TCGGGGAAAT GTGCGCGGAA  
3361 CCCCTATTTC TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC  
3421 CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTTCCGTG  
3481 TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC CAGAAACGC  
3541 TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTAC ATCGAACTGG  
3601 ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA  
3661 GCACTTTTAA AGTTCCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC  
3721 AACTCGGTCG CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG  
3781 AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA  
3841 GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG  
3901 CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA  
3961 ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAAGCAATG GCAACAACGT  
4021 TGCGCAAACCT ATTAACCTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT  
4081 GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT  
4141 TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT GCAGCACTGG  
4201 GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA  
4261 TGGATGAACG AAATAGACAG ATCGCTGAGA TAGTGCCCTC ACTGATTAAG CATTGGTAAC  
4321 TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA  
4381 AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT  
4441 TTTCTGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT  
4501 TTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT  
4561 GTTTCGCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC  
4621 AGATACCAAA TACTGTCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG  
4681 TAGCACCGCC TACATACCTC GCTCTGTCTA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG  
4741 ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT  
4801 CCGGCTGAAC GGGGGGTTTC TGCACACAG CCAGCTTGA GCGAACGACC TACACCGAAC  
4861 TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCGAAGGG AGAAAGGCGG  
4921 ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG  
4981 GAAACGCCTG GTATCTTTAT AGTCTGTCTG GGTTCGCCA CCTCTGACTT GAGCGTCTGAT  
5041 TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGAAAAA CGCCAGCAAC GCGGCCTTTT  
5101 TACGTTCTCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCTGCG TTATCCCTG  
5161 ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA  
5221 CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCTGATG CGGTATTTTC  
5281 TCCTTACGCA TCTGTGCGGT ATTTACACAC GCAGACCAGC CGCGTAACCT GGCAAAATCG  
5341 GTTACGTTG AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGGCGGA CAATAAAGTC  
5401 TTAACTGAA CAAAATAGAT CTAACTATG ACAATAAAGT CTTAACTAG ACAGAATAGT  
5461 TGTAACTGA AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGACTTT TGTATGGCT  
5521 AAAGCAAACCT CTTCAATTTT TGAAGTGCAA ATTGCCCGTC GTATTAAAGA GGGCGTGGC  
5581 CAAGGCGATG GTAAAGACTA TATTGCGGCG GTTGTGACAA TTTACCGAAC AACTCCGCGG  
5641 CCGGGAAGCC GATCTCGGCT TGAACGAATT GTTAGGTGGC GGTACTTGGG TCGATATCAA  
5701 AGTGATCAC TTCTTCCCGT ATGCCCAATG TTGTATAGAG AGCCACTGCG GGATCGTCTAC  
5761 CGTAATCTGC TTGCACGTAG ATCATAAAG CACCAAGCGC GTTGGCCTCA TGCTTGAGGA  
5821 GATTGATGAG CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGGAGACT GCGAGATCAT  
5881 AGATATAGAT CTCACTACGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC GCGAGAGCGC  
5941 CAACAACCGC TTCTTGGTCTG AAGGCAGCAA GCGCGATGAA TGTCTTACTA CGGAGCAAGT  
6001 TCCCGAGGTA ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT CCGAACTCAC  
6061 GACCGAAAAG ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGGCCG AGCCTACATG-

FIGURE 39C

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6121 TCGGAATGAT GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG CCCTGCTGCG  
6181 TAACATCGTT GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA TCGACCCACG  
6241 GCGTAACGCG CTTGCTGCTT GGATGCCCCG GGCATAGACT GTACAAAAAA ACAGTCATAA  
6301 CAAGCCATGA AAACCGCCAC TCGCGCGTTA CCACCGCTGC GTTCGGTCAA GGTTCTGGAC  
6361 CAGTTGCGTG AGCGCATACG. CTA CTACTTGTCAT TACAGTTTAC GAACCGAACA GGCTTATGTC  
6421 AACTGGGTTC GTGCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC CTTGGGCAGC  
6481 AGCGAAGTCG AGGCATTTCT GTCCTGGCTG GCGAACGAGC GCAAGGTTTC GGTCTCCACG  
6541 CATCGTCAGG CATTGGCGGC CTTGCTGTTC TTCTACGGCA AGGTGCTGTG CACGGATCTG  
6601 CCCTGGCTTC AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGCCGGT GGTGCTGACC  
6661 CCGGATGA

FIGURE 39A

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Figure 40A: pDEST20 Glutathione-S-transferase Fusion with Polyhedron Promoter for Baculovirus Expression

430 ggc tac gta tac tcc gga ata tta ata gat cat gga gat aat taa aat gat  
ccg atg cat atg agg cct tat aat tat cca gta cct cta tta att tta cta

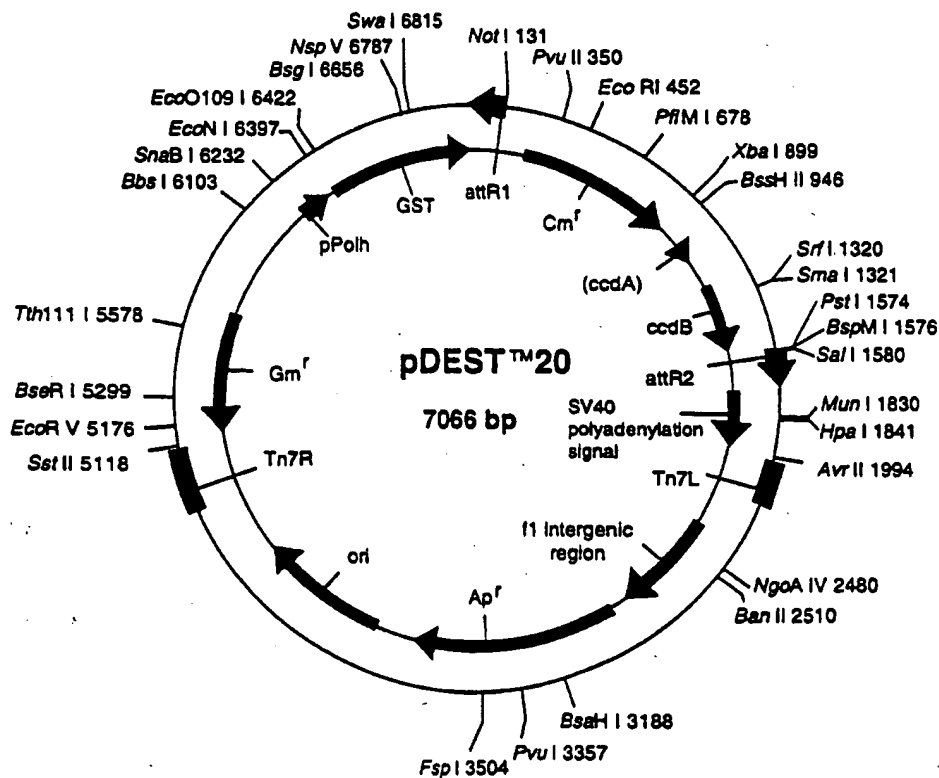
481 aac cat ctc gca aat aaa taa gta ttt tac tgt ttt cgt aac agt ttt gta  
ttg gta gag cgt tta ttt att cat aaa atg aca aaa gca ttg tca aaa cat

532 ata aaa aaa cct ata aat att ccg gat tat tca tac cgt ccc acc atc ggg  
tat ttt ttt gga tat tta taa ggc cta ata agt atg gca ggg tgg tag ccc

Start Transl. M A P T - - GST - -  
583 cgc gga tcc atg gcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg  
gcg cct agg tac cgg gga tat gat cca ata acc ttt taa ttc ccg gaa cac

1246 S D L V P R H N Q T S L Y K K A  
cgc gat ctg gtt ccg cgt cat aat caa aca agt ttg tac aaa aaa gct gaa  
agc cta gac caa ggc gca gta tta gtt tgt tca aac atg ttt ttt cga ctt

1297 cga gaa acg taa aat gat ata aat atc aat ata tta aat tag at  
gct ctt tgc att tta cta tat tta tag tta tat aat tta atc ta



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**pDEST20 7066 bp (rotated to position 5800)**

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
592..1263		GST
1397..1273		attR1
1506..2165		CmR
2285..2369		inactivated ccdA
2507..2812		ccdB
2853..2977		attR2
4214..5064		ampR
5263..5843		ori

1	CCACTGCGCC	GTTACCACCG	CTGCGTTCCG	TCAAGGTTCT	GGACCAAGTTG	CGTGAGCGCA
61	TACGCTACTT	GCATTACAGT	TTACGAACCG	AACAGGCTTA	TGTCAACTGG	GTTTCGTGCCT
121	TCATCCGTTT	CCACGGTGTG	CGTCACCCGG	CAACCTTGGG	CAGCAGCGAA	GTCGAGGCAT
181	TTCTGTCTCG	GCTGGCGAAC	GAGCGCAAGG	TTTCGGTCTC	CACGCATCGT	CAGGCATTGG
241	CGGCCCTTGCT	GTTCTTCTAC	GGCAAGGTGC	TGTGCACGGA	TCTGCCCTGG	CTTCAGGAGA
301	TCGGAAGACC	TCGGCCGTCG	CGGCGCTTGC	CGGTGGTGCT	GACCCCGGAT	GAAGTGGTTC
361	GCATCCTCGG	TTTTCTGGAA	GGCGAGCATC	GTTTGTTCGC	CCAGGACTCT	AGCTATAGTT
421	CTAGTGGTTG	GCTACGTATA	CTCCGGAATA	TTAATAGATC	ATGGAGATAA	TTAAAATGAT
481	AACCATCTCG	CAAATAAATA	AGTATTTTAC	TGTTTTTCGTA	ACAGTTTTGT	AATAAAAAAA
541	CCTATAAATA	TTCCCGATTA	TTCATACCGT	CCCACCATCG	GGCGCGGATC	CATGGCCCCCT
601	ATACTAGGTT	ATTGGAAAAT	TAAGGGCCTT	GTGCAACCCA	CTCGACTTCT	TTTGAATAT
661	CTTGAAGAAA	AATATGAAGA	GCATTTGTAT	GAGCGCGATG	AAGGTGATAA	ATGGCGAAAC
721	AAAAAGTTTG	AATTGGGTTT	GGAGTTTCCC	AATCTTCCTT	ATTATATTGA	TGGTGATGTT
781	AAATTAACAC	AGTCTATGGC	CATCATACTG	TATATAGCTG	ACAAGCACAA	CATGTTGGGT
841	GGTTGTCCAA	AAGAGCGTGC	AGAGATTTCA	ATGCTTGAAG	GAGCGGTTTT	GGATATTAGA
901	TACGGTGTTC	CGAGAAATGC	ATATAGTAAA	GACTTTGAAA	CTCTCAAAGT	TGATTTTCTT
961	AGCAAGCTAC	CTGAAATGCT	GAAAATGTTT	GAAGATCGTT	TATGTCATAA	AACATATTTA
1021	AATGGTGATC	ATGTAACCCA	TCCTGACTTC	ATGTTGTATG	ACGCTCTTGA	TGTTGTTTTA
1081	TACATGGACC	CAATGTGCCT	GGATGCGTTC	CCAAAATTAG	TTTGTTTTAA	AAAACGTATT
1141	GAAAGCTATC	CACAAATTGA	TAAGTACTTG	AAATCCAGCA	AGTATATAGC	AATGCCTTTG
1201	CAGGGCTGGC	AAGCCACGTT	TGGTGGTGGC	GACCATCCTC	CAAAATCGGA	TCTGGTTCCT
1261	CGTCATAATC	AAACAAGTTT	GTACAAAAAA	GCTGAACGAG	AAACGTAAAA	TGATATAAAT
1321	ATCAATATAT	TAAATTAGAT	TTTGCAATAA	AAACAGACTA	CATAAATACT	TAAACACAAA
1381	CATATCCAGT	CACTATGGCG	GCCGCATTAG	GCACCCAGG	CTTTACACTT	TATGCTTCCG
1441	GCTCGTATGT	TGTGTGGATT	TTGAGTTAGG	ATCCGGCGAG	ATTTTCAGGA	GCTAAGGAAG
1501	CTAAAATGGA	GAAAAAATC	ACTGGATATA	CCACCGTTGA	TATATCCCAA	TGGCATCGTA
1561	AAGAACATTT	TGAGGCATTT	CAGTCAGTTG	CTCAATGTAC	CTATAACCAG	ACCGTTCAGC
1621	TGGATATTAC	GGCCTTTTAA	AAGACCGTAA	AGAAAAATAA	GCACAAGTTT	TATCCGGCCT
1681	TTATTCACAT	TCTTGCCCGC	CTGATGAATG	CTCATCCGGA	ATTCCGTATG	GCAATGAAAG
1741	ACGGTGAGCT	GGTGATATGG	GATAGTGTTT	ACCCTTGTTA	CACCGTTTTT	CATGAGCAAA
1801	CTGAAACGTT	TTCATCGCTC	TGGAGTGAAT	ACCACGACGA	TTTCCGGCAG	TTTCTACACA
1861	TATATTCGCA	AGATGTGGCG	TGTTACGGTG	AAAACCTGGC	CTATTTCCCT	AAAGGGTTTA
1921	TTGAGAATAT	GTTTTTCGTC	TCAGCCAATC	CCTGGGTGAG	TTTCACCACT	TTTGATTTAA
1981	ACGTGGCCAA	TATGGACAAC	TTCTTCGCCC	CCGTTTTTCAC	CATGGGCAAA	TATTATACGC
2041	AAGGCGACAA	GGTGCTGATG	CCGCTGGCGA	TTCAAGTTCA	TCATGCCGTC	TGTGATGGCT
2101	TCCATGTCGG	CAGAATGCTT	AATGAATTAC	AACAGTACTG	CGATGAGTGG	CAGGGCGGGG
2161	CGTAATCTAG	AGGATCCGGC	TTACTAAAAG	CCAGATAACA	GTATGCGTAT	TTGCGCGCTG
2221	ATTTTTGCGG	TATAAGAATA	TATACTGATA	TGTATACCCG	AAGTATGTCA	AAAAGAGGTG
2281	TGCTATGAAG	CAGCGTATTA	CAGTGACAGT	TGACAGCGAC	AGCTATCAGT	TGCTCAAGGC
2341	ATATATGATG	TCAATATCTC	CGGTCTGGTA	AGCACAACCA	TGCAGAAATGA	AGCCCCGCTG
2401	CTGCGTGGCG	AACGCTGGAA	AGCGGAAAGT	CAGGAAGGGA	TGGCTGAGGT	CGCCCCGTTT
2461	TTTGAAATGA	ACGGCTCTTT	TGCTGACGAG	AACAGGGACT	GGTGAAATGC	AGTTTAAGGT
2521	TTACACCTAT	AAAAGAGAGA	GCCGTTATCG	TCTGTTTGTG	GATGTACAGA	GTGATATTAT
2581	TGACACGCCC	GGGCGACGGA	TGGTGATCCC	CCTGGCCAGT	GCACGTCTGC	TGTCAGATAA
2641	AGTCTCCCGT	GAACCTTACC	CGGTGGTGCA	TATCGGGGAT	GAAAGCTGGC	GCATGATGAC

Figure 40B



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2701 CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA GAAGTGGCTG ATCTCAGCCA  
2761 CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTC TGGGGAATAT AAATGTCAGG  
2821 CTCCCTTATA CACAGCCAGT CTGCAGGTCG ACCATAGTGA CTGGATATGT TGTGTTTTAC  
2881 AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTAA TATATTGATA TTTATATCAT  
2941 TTTACGTTTC TCGTTCAGCT TTCTTGACAA AAGTGGTTTG ATAGCTTGTC GAGAAGTACT  
3001 AGAGGATCAT AATCAGCCAT ACCACATTTG TAGAGGTTTT ACTTGCTTTA AAAAACCTCC  
3061 CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTTGTTGTT AACTTGTTTA  
3121 TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTCACA AATAAAGCAT  
3181 TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAATCAT CAATGTATCT TATCATGTCT  
3241 GGATCTGATC ACTGCTTGAG CCTAGGAGAT CCGAACCAGA TAAGTGAAAT CTAGTTCCAA  
3301 ACTATTTTGT CATTTTAAAT TTTCGTATTA GCTTACGACG CTACACCCAG TTCCCATCTA  
3361 TTTTGTCACT CTTCCCTAAA TAATCCTTAA AAATCCATT TCCACCCCTC CCAGTTCCCA  
3421 ACTATTTTGT CCGCCACAG CGGGGCATTT TTCTTCTGT TATGTTTTTA ATCAAACATC  
3481 CTGCCAATC CATGTGACAA ACCGTCATCT TCGGCTACTT TTTCTCTGTC ACAGAATGAA  
3541 AATTTTTCTG TCATCTCTTC GTTATTAATG TTGTAAATG ACTGAATATC AACGCTTATT  
3601 TGCAGCCTGA ATGGCGAATG GACGCGCCCT GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG  
3661 TGGTTACGCG CAGCGTGACC GCTACACTTG CCAGCGCCCT AGCGCCGCTT CTTTCCGCTT  
3721 TCTTCCCTTC CTTTCTCGCC ACGTTCGCGG GCTTCCCGG TCAAGCTCTA AATCGGGGGC  
3781 TCCCTTTAGG GTTCCGATTT AGTGCTTTAC GGCACCTCGA CCCCCAAAAA CTTGATTAGG  
3841 GTGATGGTTC ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCCT TTGACGTGG  
3901 AGTCCACGTT CTTTAATAGT GGACTCTTGT TCCAACTGG AACAACATC AACCCTATCT  
3961 CGGTCTATTC TTTTGATTTA TAAGGATTT TGCCGATTTT GGCCTATTGG TTAATAATG  
4021 AGCTGATTTA ACAAAAATTT AACGCGAATT TTAACAAAAT ATTAACGTTT ACAATTTTCA  
4081 GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTG TTTATTTTTT TAAATACATT  
4141 CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA  
4201 GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT  
4261 GCCTTCCTGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT  
4321 TGGGTGCACG AGTGGGTTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT  
4381 TTCGCCCCGA AGAACGTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG  
4441 TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTG CCGCATACAC TATTCTCAGA  
4501 ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA  
4561 GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TACTTCTGA  
4621 CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA  
4681 CTCGCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA  
4741 CCACGATGCC TGTAGCAATG GCAACAACGT TGCGCAACT ATTAAGTGGC GAACTACTTA  
4801 CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC  
4861 TTCTGCGCTC GGCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC  
4921 GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG  
4981 TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA  
5041 TAGGTGCTTC ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT  
5101 AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA  
5161 ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTTCGTTCCA CTGAGCGTCA GACCCCGTAG  
5221 AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA  
5281 CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT  
5341 TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATAACCAA TACTGTCTTT CTAGTGTAGC  
5401 CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA  
5461 TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA  
5521 GACGATAGTT ACCGATAAG GCGCAGCGGT CGGGCTGAAC GGGGGGTTCTG TGCACACAGC  
5581 CCAGCTTGA GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA  
5641 GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA  
5701 CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCCTG GTATCTTTAT AGTCTGTGCG  
5761 GGTTCGCGCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC  
5821 TATGGA AAAA CGCCAGCAAC GCGGCCCTTT TACGGTTTCT GGCCTTTTGG TGGCCTTTTG  
5881 CTCACATGTT CTTTCTGCG TTATCCCTGA ATTCTGTGGA TAACCTTATT ACCGCTTTG  
5941 AGTGAGCTGA TACCGTTCG CCGACGCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG  
6001 AAGCGGAAGA GCGCCTGATG CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACAC  
6061 GCAGACCAGC CGCGTAACCT GGCAAAATCG GTTACGGTTG AGTAATAAAT GGATGCCCTG  
6121 CGTAAGCGGG TGTGGGCGGA CAATAAAGTC TTAAGTGA CAAAATAGAT CTAACTATG-

Figure 40C

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6181 ACAATAAAGT CTTAAACTAG ACAGAATAGT TGTAAGTGA AATCAGTCCA GTTATGCTGT  
6241 GAAAAAGCAT ACTGGACTTT TGTTATGGCT AAAGCAAACCT CTTCAATTTTC TGAAGTGCAA  
6301 ATTGCCCCGTC GTATTAAAGA GGGGCGTGGC CAAGGGCATG GTAAAGACTA TATTCGCGGC  
6361 GTTGTGACAA TTTACCGAAC AACTCCGCGG CCGGGAAGCC GATCTCGGCT TGAACGAATT  
6421 GTTAGGTGGC GGTACTTGGG TCGATATCAA AGTGATCAC TTTCTCCCGT ATGCCCAACT  
6481 TTGTATAGAG AGCCACTGCG GGATCGTCAC CGTAATCTGC TTGCACGTAG ATCACATAAG  
6541 CACCAAGCGC GTTGGCCTCA TGCTTGAGGA GATTGATGAG CGCGGTGGCA ATGCCCTGCC  
6601 TCCGGTGCTC GCCGGAGACT GCGAGATCAT AGATATAGAT CTCACTACGC GGCTGCTCAA  
6661 ACCTGGGCAG AACGTAAGCC GCGAGAGCGC CAACAACCGC TTCTTGGTCG AAGGCAGCAA  
6721 GCGCGATGAA TGTCTTACTA CGGAGCAAGT TCCCGAGGTA ATCGGAGTCC GGCTGATGTT  
6781 GGGAGTAGGT GGCTACGTCT CCGAACTCAC GACCGAAAAG ATCAAGAGCA GCCCGCATGG  
6841 ATTTGACTTG GTCAGGGCCG AGCCTACATG TGCGAATGAT GCCCATACTT GAGCCACCTA  
6901 ACTTTGTTTT AGGGCGACTG CCCTGCTGCG TAACATCGTT GCTGCTGCGT AACATCGTTG  
6961 CTGCTCCATA ACATCAAACA TCGACCCACG GCGTAACGCG CTTGCTGCTT GGATGCCCGA  
7021 GGCATAGACT GTACAAAAA ACAGTCATAA CAAGCCATGA AAACCG

FIGURE 40D

Figure 41A:

pDEST21

**2-Hybrid Vector with  
DNA-Binding Domain**

**ADH Promoter**

700 ~~ttg gcg ctt tgc tat caa gta taa ata gac ctg caa tta tca atc ttc tgt~~  
~~aac ggc gaa acg ata gtt cat att tat ctg ggc gtt aat aat tag aaa aca~~

751 ~~ttc ctc gtc att gtt ctc gtt ccc ttt ctt cct tgt ttc ttt ttc tgc aca~~  
~~aac gag cag taa caa gaa caa gaa aaa gaa gga aca gag aaa aag acg tgt~~

802 ~~ata ttt caa gct ata cca agc ata caa tca act~~ cca agc ttg aag caa gcc  
~~tat aaa ggt cga tat ggt tgc tat gtt aat tga~~ ggt tgc aac ttc gtt cgg

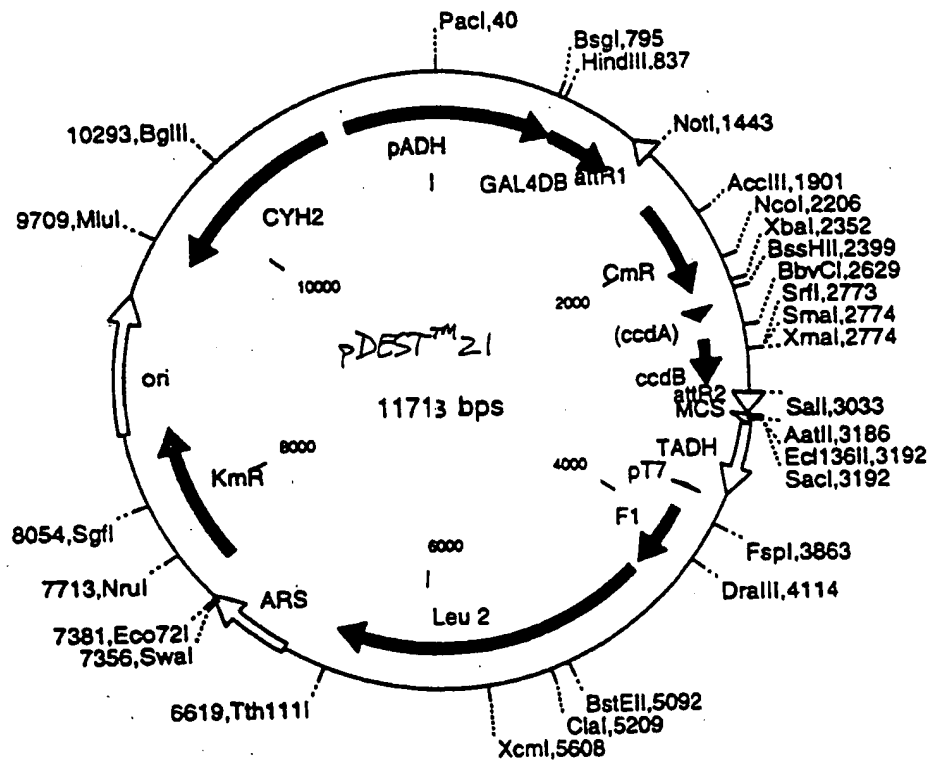
853 ~~ttc tga aag atg aag cta ctg tct tct atc gaa caa gca tgc gat att tgc~~  
~~agg act ttc tac ttc gat gac aga tag ctt gtt cgt acg cta taa acg~~

1261 gaa gag agt agt aac aaa ggt caa aga cag ttg act gta tgg tgg agg tgg  
ctt ctc tca tca ttg ttt cca gtt tct gtc aac tga cat agc agc tcc agc

1312 ~~aca agt tgg tac aaa aaa gct gaa cga gaa acg taa aat gat ata~~  
~~tca gtt tgt tca aac atg ttt ttt cga ctt gct ctt tgc att tta cta tat~~

Start Transl. M K L L S S Gal4-DB  
T S L Y K K A acc R1

ZNF V



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## pDEST21 11713 bp (rotated to position 11000)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
857..1322		GAL4DB
1456..1332		attR1
1706..2365		CmR
2485..2569		inactivated ccdA
2707..3012		ccdB
3053..3177		attR2
3716..3735		pT7 (T7 promoter)
3899..4354		f1 (f1 intergenic region)
4414..6642		Leu2
7541..8515		kanR
9668..10958		CYH2
11118..848		pADH (ADH promoter)

1	TTTATTATGT	TACAATATGG	AAGGGAACCT	TACACTTCTC	CTATGCACAT	ATATTAATTA
61	AAGTCCAATG	CTAGTAGAGA	AGGGGGGTAA	CACCCCTCCG	CGCTCTTTTC	CGATTTTTTT
121	CTAAACCGTG	GAATATTTCG	GATATCCTTT	TGTTGTTTCC	GGGTGTACAA	TATGGACTTC
181	CTCTTTTCTG	GCAACCAAAC	CCATACATCG	GGATTCCTAT	AATACCTTCG	TTGGTCTCCC
241	TAACATGTAG	GTGGCGGAGG	GGAGATATAC	AATAGAACAG	ATACCAGACA	AGACATAATG
301	GGCTAAACAA	GACTACACCA	ATTACACTGC	CTCATTGATG	GTGGTACATA	ACGAACTAAT
361	ACTGTAGCCC	TAGACTTGAT	AGCCATCATC	ATATCGAAGT	TTCACTACCC	TTTTTCCATT
421	TGCCATCTAT	TGAAGTAATA	ATAGGCGCAT	GCAACTTCTT	TTCTTTTCTT	TTCTTTTCTC
481	TCTCCCCCGT	TGTTGTCTCA	CCATATCCGC	AATGACAAAA	AAAATGATGG	AAGACACTAA
541	AGGAAAAAAT	TAACGACAAA	GACAGCACCA	ACAGATGTCG	TTGTTCCAGA	GCTGATGAGG
601	GGTATCTTCG	AACACACGAA	ACTTTTTCTT	TCCTTCATTC	ACGCACACTA	CTCTCTAATG
661	AGCAACGGTA	TACGGCCTTC	CTTCCAGTTA	CTTGAATTGG	AAATAAAAAA	AGTTTGCCGC
721	TTTGCTATCA	AGTATAAATA	GACCTGCAAT	TATTAATCTT	TTGTTTCCTC	GTCATTGTTC
781	TCGTTCCCTT	TCTTCCTTGT	TTCTTTTTCT	GCACAATATT	TCAAGCTATA	CCAAGCATAC
841	AATCAACTCC	AAGCTTGAAG	CAAGCCTCCT	GAAAGATGAA	GCTACTGTCT	TCTATCGAAC
901	AAGCATGCGA	TATTTGCCGA	CTTAAAAAGC	TCAAGTGCTC	CAAAGAAAAA	CCGAAGTGCG
961	CCAAGTGTCT	GAAGAACAAC	TGGGAGTGTC	GCTACTCTCC	CAAAACCAAA	AGGTCTCCGC
1021	TGACTAGGGC	ACATCTGACA	GAAGTGGAAT	CAAGGCTAGA	AAGACTGGAA	CAGCTATTTT
1081	TACTGATTTT	TCCTCGAGAA	GACCTTGACA	TGATTTTGAA	AATGGATTCT	TTACAGGATA
1141	TAAAAGCATT	GTTAACAGGA	TTATTTGTAC	AAGATAATGT	GAATAAAGAT	GCCGTCACAG
1201	ATAGATTGGC	TTCAAGTGGG	ACTGATATGC	CTCTAACATT	GAGACAGCAT	AGAATAAGTG
1261	CGACATCATC	ATCGGAAGAG	AGTAGTAACA	AAGGTCAAAG	ACAGTTGACT	GTATCGTCGA
1321	GGTCGAATCA	AACAAGTTTG	TACAAAAAAG	CTGAACGAGA	AACGTAATAA	GATATAAATA
1381	TCAATATATT	AAATTAGATT	TTGCATAAAA	AACAGACTAC	ATAATACTGT	AAAACACAAC
1441	ATATCCAGTC	ACTATGGCGG	CCGCTAAGTT	GGCAGCATCA	CCCGACGCAC	TTTGCGCCGA
1501	ATAAATACCT	GTGACGGAAG	ATCACTTCGC	AGAATAAATA	AATCCTGGTG	TCCCTGTGTA
1561	TACCGGGAAG	CCCTGGGCCA	ACTTTTGGCG	AAAATGAGAC	GTTGATCGGC	ACGTAAGAGG
1621	TTCCAACCTT	CACCATAATG	AAATAAGATC	ACTACCGGGC	GTATTTTTTG	AGTTATCGAG
1681	ATTTTTCAGGA	GCTAAGGAAG	CTAAAAATGGA	GAAAAAATC	ACTGGATATA	CCACCGTTGA
1741	TATATCCCAA	TGGCATCGTA	AAGAACATTT	TGAGGCATTT	CAGTCAGTTG	CTCAATGTAC
1801	CTATAACCAG	ACCGTTCAGC	TGGATATTAC	GGCCTTTTTA	AAGACCGTAA	AGAAAAATAA
1861	GCACAAGTTT	TATCCGGCCT	TTATTCACAT	TCTTGCCCGC	CTGATGAATG	CTCATCCGGA
1921	ATTCCGTATG	GCAATGAAAG	ACGGTGAGCT	GGTGATATGG	GATAGTGTTT	ACCTTGTTTA
1981	CACCGTTTTT	CATGAGCAAA	CTGAAACGTT	TTATCCTGTC	TGGAGTGAAT	ACCACGACGA
2041	TTTCCGGCAG	TTTCTACACA	TATATTCGCA	AGATGTGGCG	TGTTACGGTG	AAAACCTGGC
2101	CTATTTCCCT	AAAGGGTTTA	TTGAGAATAT	GTTTTTCGTC	TCAGCCAATC	CCCTGGGTGAG
2161	TTTCACCAGT	TTTGATTATA	ACGTGGCCAA	TATGGACAAC	TTCTTCGCCC	CCGTTTTTCAC
2221	CATGGGCAAA	TATTATACGC	AAGGCGACAA	GGTGCTGATG	CCGCTGGCGA	TTCAAGTTCA
2281	TCATGCCGTC	TGTGATGGCT	TCCATGTCGG	CAGAATGCTT	AATGAATTAC	AACAGTACTG
2341	CGATGAGTGG	CAGGGCGGGG	CGTAATCTAG	AGGATCCGGC	TTACTAAAAG	CCAGATAACA
2401	GTATGCGTAT	TTGCGCGCTG	ATTTTTGCGG	TATAAGAATA	TATACTGATA	TGTATACCGG-

FIGURE 413

2461 AAGTATGTCA AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT TGACAGCGAC  
2521 AGCTATCAGT TGCTCAAGGC ATATATGATG TCAATATCTC CGGTCTGGTA AGCACAACCA  
2581 TGCAGAAATGA AGCCCCGTCG CTGCGTGCCG AACGCTGGAA AGCGGAAAAT CAGGAAGGGA  
2641 TGGCTGAGGT CGCCCCGTTT ATTGAAATGA ACGGCTCTTT TGCTGACGAG AACAGGGACT  
2701 GGTGAAATGC AGTTTAAAGT TTACACCTAT AAAAGAGAGA GCCGTTATCG TCTGTTTGTG  
2761 GATGTACAGA GTGATATTAT TGACACGCCC GGGCGACGGA TGGTGATCCC CCTGGCCAGT  
2821 GCACGCTGTC TGTCAGATAA AGTCTCCCGT GAACTTTACC CGGTGGTGCA TATCGGGGAT  
2881 GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA  
2941 GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTC  
3001 TGGGGAATAT AAATGTCAGG CTCCCTTATA CACAGCCAGT CTGCAGGTCG ACCATAGTGA  
3061 CTGGATATGT TGTGTTTTAC AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTAA  
3121 TATATTGATA TTTATATCAT TTTACGTTTC TCGTTCAGCT TTCTTGTAACA AAGTGGTTTG  
3181 ATGGCCGCTA AGTAAGTAAG ACGTCGAGCT CTAAGTAAGT AACGGCCGCC ACCGCGGTGG  
3241 AGCTTTGGAC TTCTTCGCCA GAGGTTTGGT CAAGTCTCCA ATCAAGGTTG TCGGCTTGTC  
3301 TACCTTGCCA GAAATTTACG AAAAGATGGA AAAGGGTCAA ATCGTTGGTA GATACGTTGT  
3361 TGACACTTCT AAATAAGCGA ATTTCTTATG ATTTATGATT TTTATTATTA AATAAGTTAT  
3421 AAAAAAATA AGTGATATAA AATTTAAAG TGACTCTTAG GTTTTAAAC GAAAATCTT  
3481 ATTCTTGAGT AACTCTTCC TGTAGGTCAG GTTGCTTTCT CAGGTATAGC ATGAGGTCGC  
3541 TCTTATTGAC CACACCTCTA CCGGCATGCC GAGCAAATGC CTGCAAATCG CTCCCCATTT  
3601 CACCCAATTG TAGATATGCT AACTCCAGCA ATGAGTTGAT GAATCTCGGT GTGTATTTTA  
3661 TGTCCTCAGA GGACAATACC TGTTGTAATC GTTCTTCCAC ACGGATCCCA ATTCGCCCTA  
3721 TAGTGAGTCG TATTACAATT CACTGGCCGT CGTTTACAA CGTCGTGACT GGGAAAACCC  
3781 TGGCGTTACC CAACTTAATC GCCTTGACG ACATCCCCCT TTCGCCAGCT GCGGTAATAG  
3841 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG GCGAATGGAC  
3901 GCGCCCTGTA GCGGCGCATT AAGCGCGCG GGTGTGGTGG TTACGCGCAG CGTGACCGCT  
3961 ACACTTGCCA GCGCCCTAGC GCCCGTCTCT TCGCTTTCT TCCCTTCCTT TCTCGCCACG  
4021 TTCGCCGGCT TTCCCCGTC AAGCTCTAAAT CGGGGGCTCC CTTAGGGTT CCGATTTAGT  
4081 GCTTTACGGC ACCTCGACCC CAAAAAATT GATTAGGGTG ATGGTTCAG TAGTGGGCCA  
4141 TCGCCCTGAT AGACGGTTTT TCGCCCTTTG ACGTTGGAGT CCACGTTCTT TAATGTTGA  
4201 CTCTTGTTCC AAACCTGGAAC AACACTCAAC CCTATCTCGG TCTATTCTTT TGATTTATAA  
4261 GGGATTTTGC CGATTTCGGC CTATTGTTA AAAAATGAGC TGATTTAACA AAAATTTAAC  
4321 GCGAATTTTA ACAAATATT AACGTTTACA ATTTCTGAT GCGGTATTTT CTCCTTACGC  
4381 ATCTGTGCGG TATTTACAC CGCATATCGA CCGTTCGAGG AGAATTCTA GTATATCCAC  
4441 ATACCTAATA TTATTGCCTT ATTAATAATG GAATCGGAAC AATTACATCA AAATCCACAT  
4501 TCTCTTCAA ATCAATTGTC CTGTACTTCC TTGTTTCATG GTGTTCAAAA ACGTTATATT  
4561 TATAGGATAA TTATACTCTA TTTCTCAACA AGTAATTGGT TGTTTGGCCG AGCGGTCTAA  
4621 GCGCCTGAT TCAAGAAATA TCTTGACCGC AGTTAACTGT GGGAAATACTC AGGTATCGTA  
4681 AGATGCAAGA GTTCGAATCT CTTAGCAACC ATTATTTTTT TCCTCAACAT AACGAGAACA  
4741 CACAGGGGCG CTATCGCACA GAATCAAATT CGATGACTGG AAATTTTTTG TTAATTTAG  
4801 AGGTGCGCTG ACGCATATAC CTTTTTCAAC TGAAAAATTG GGAGAAAAAG GAAAGGTGAG  
4861 AGGCCGGAAC CGGCTTTTCA TATAGAATAG AGAAGCGTTC ATGACTAAAT GCTTGCATCA  
4921 CAATACTTGA AGTTGACAAT ATTATTTAAG GACCTATTGT TTTTCCAAT AGGTGGTTAG  
4981 CAATCGTCTT ACTTTCTAAC TTTTCTTACC TTTTACATTT CAGCAATATA TATATATATT  
5041 TCAAGGATAT ACCATTCTAA TGTCTGCCCC TATGTCTGCC CTAAGAAGA TCGTCGTTTT  
5101 GCCAGGTGAC CACGTTGGTC AAGAAATCAC AGCCGAAGCC ATTAAGGTTT TAAAGCTAT  
5161 TTCTGATGTT CGTTCCAATG TCAAGTTCGA TTTCGAAAAT CATTTAATTG GTGGTGCTGC  
5221 TATCGATGCT ACAGGTGTCC CACTTCCAGA TGAGGCGCTG GAAGCCTCCA AGAAGGTTGA  
5281 TGCCGTTTTG TTAGGTGCTG TGGGTGGTCC TAAATGGGGT ACCGGTAGTG TTAGACCTGA  
5341 ACAAGGTTTA CTAATAATCC GTAAAGAACT TCAATTGTAC GCCAACTTAA GACCATGTAA  
5401 CTTTGTCATCC GACTCTCTTT TAGACTTATC TCCAATCAAG CCACAATTTG CTAAGGTAC  
5461 TGACTTCGTT GTTGTCAGAG AATTAGTGGG AGGTATTTAC TTTGGTAAGA GAAAGGAAGA  
5521 CGATGGTGAT GGTGTGCTT GGGATAGTGA ACAATACACC GTTCCAGAAG TGCAAGAAT  
5581 CACAAGAATG GCCGCTTTCA TGGCCCTACA ACATGAGCCA CCATTGCCTA TTTGGTCTT  
5641 GGATAAAGCT AATGTTTTGG CCTCTTCAAG ATTATGGAGA AAAACTGTGG AGGAAACCAT  
5701 CAAGAACGAA TTCCCTACAT TGAAGGTTCA ACATCAATTG ATTGATCTG CCGCATGAT  
5761 CCTAGTTAAG AACCCAACCC ACCTAAATGG TATTATAATC ACCAGCAACA TGTTTGGTGA  
5821 TATCATCTCC GATGAAGCCT CCGTTATCCC AGGTTCCTTG GGTGTTGTGC CATCTGCGTC  
5881 CTTGGCCTCT TTGCCAGACA AGAACACCGC ATTTGGTTTG TACGAACCAT GCCACGGTTC-

Figure 41C

5941 TGCTCCAGAT TTGCCAAAGA ATAAGGTTGA CCTATCGCC ACTATCTTGT CTGCTGCAAT  
6001 GATGTTGAAA TTGTCATTGA ACTTGCCTGA AGAAGGTAAG GCCATTGAAG ATGCAGTTAA  
6061 AAAGGTTTTG GATGCAGGTA TCAGAACTGG TGATTTAGGT GGTTCACAAC GTACCACCGA  
6121 AGTCGGTGAT GCTGTCGCCG AAGAAGTTAA GAAAATCCTT GCTTAAAAAG ATTCTCTTTT  
6181 TTTATGATAT TTGTACATAA ACTTTATAAA TGAAATTCAT AATAGAAACG ACACGAAATT  
6241 ACAAATGGA ATATGTTTCA AGGGTAGACG AAACATATA CGCAATCTAC ATACATTTAT  
6301 CAAGAAGGAG AAAAAGGAGG ATAGTAAAGG AATACAGGTA AGCAAATTGA TACTAATGGC  
6361 TCAACGTGAT AAGGAAAAAG AATTGCACCT TAACATTAAT ATTGACAAGG AGGAGGGCAC  
6421 CACACAAAAA GTTAGGTGTA ACAGAAAATC ATGAAACTAC GATTCCTAAT TTGATATTGG  
6481 AGGATTTTCT CTAAAAAAA AAAAATACAA CAAATAAAAA ACACTCAATG ACCTGACCAT  
6541 TTGATGGAGT TTAAGTCAAT ACCTTCTTGA ACCATTTCCC ATAATGGTGA AAGTTCCCTC  
6601 AAGAATTTTA CTCTGTCAGA AACGGCCTTA CGACGTAGTC GATATGGTGC ACTCTCAGTA  
6661 CAATCTGCTC TGATGCCGCA TAGTTAAGCC AGCCCCGACA CCCGCCAACA CCCGCTGACG  
6721 CGCCCTGACG GGCTTGTCTG CTCCCGGCAT CCGCTTACAG ACAAGCTGTG ACCGCTCTCG  
6781 GGAGCTGCAT GTGTCAGAGG TTTTCACCGT CATCACCGAA ACGCGCGAGA CGAAAGGGCC  
6841 TCGTGATACG CCTATTTTTA TAGGTTAATG TCATGATAAT AATGGTTTCT TAGGACGGAT  
6901 CGCTTGCCCTG TAACCTACAC GCGCCTCGTA TCTTTTAAATG ATGGAATAAT TTGGGAATTT  
6961 ACTCTGTGTT TATTTATTTT TATGTTTTGT ATTTGGATTT TAGAAAGTAA ATAAAGAAGG  
7021 TAGAAGAGTT ACGGAATGAA GAAAAAATAA TAAACAAAGG TTTAAAAAAT TTCAACAAAA  
7081 AGCGTACTTT ACATATATAT TTATTAGACA AGAAAAGCAG ATTAATAGTA TATACATTCTG  
7141 ATTAACGATA AGTAAAATGT AAAATCACAG GATTTTCGTG TGTGGTCTTC TACACAGACA  
7201 AGATGAAACA ATTCGGCATT AATACCTGAG AGCAGGAAGA GCAAGATAAA AGGTAGTATT  
7261 TGTTGCGGAT CCCCTAGAG TCTTTTACAT CTTTCGAAAA CAAAAACTAT TTTTCTTTTA  
7321 ATTTCTTTTT TTACTTTCTA TTTTAAATTT ATATATTTAT ATTAAAAAAT TTAAATTATA  
7381 ATTATTTTTA TAGCACGTGA TGAAAAGGAC CCAGGTGGCA CTTTTCGGGG AAATGTGCGC  
7441 GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA  
7501 TAACCTGAT AAATGCTTCA ATAATCTGCA GCTCTGGCCC GTGTCTCAA ATCTCTGATG  
7561 TTACATTGCA CAAGATAAAA ATATATCATC ATGAACAATA AAACGTGTCTG CTTACATAAA  
7621 CAGTAATACA AGGGGTGTTA TGAGCCATAT TCAACGGGAA ACGTCTTGCT GGAGGCCGCG  
7681 ATTAATTTCC AACATGGATG CTGATTTTATA TGGGTATAAA TGGGCTCGCG ATAATGTCTG  
7741 GCAATCAGGT GCGACAATCT TTCGATTGTA TGGGAAGCCC GATGCGCCAG AGTTGTTTCT  
7801 GAAACATGGC AAAGGTAGCG TTGCCAATGA TGTTACAGAT GAGATGGTCA GACTAAACTG  
7861 GCTGACGGAA TTTATGCCTC TTCCGACCAT CAAGCATTTT ATCCGTACTC CTGATGATGC  
7921 ATGGTTACTC ACCACTGCGA TCCGCGGGAA AACAGCATT CAGGTATTAG AAGAATATCC  
7981 TGATTCAAGT GAAAATATTG TTGATGCGCT GGCAGTGTTC CTGCGCCGGT TGCATTGAT  
8041 TCCTGTTTGT AATTGTCTTT TTAACAGCGA TCGCGTATTT CGTCTCGCTC AGGCGCAATC  
8101 ACCGAATGAAT AACGGTTTGG TTGATGCGAG TGATTTTGAT GACGAGCGTA ATGGCTGGCC  
8161 TGTTGAACAA GTCTGGAAG AAATGCATAC GCTTTTGCCA TTCTACCCGG ATTCAGTCGT  
8221 CACTCATGGT GATTTCTCAC TTGATAACCT TATTTTGTAC GAGGGGAAAT TAATAGGTTG  
8281 TATTGATGTT GGACGAGTCG GAATCGCAGA CCGATACCAG GATCTTGCCA TCCTATGGAA  
8341 CTGCCTCGGT GAGTTTTCTC CTTCAATTACA GAAACGGCTT TTTCAAAAA ATGGTATTGA  
8401 TAATCCTGAT ATGAATAAAT TGCAGTTTCA TTTGATGCTC GATGAGTTTT TCTAATCAGA  
8461 ATTGGTTAAT TGGTTGTAAC ACTGGCAGAG CATTACGCTG ACTTGACGGG ACGGCGCATG  
8521 ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC  
8581 AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA  
8641 CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG  
8701 GTAACCTGGT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA  
8761 GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA  
8821 CCACTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGACTC AAGACGATAG  
8881 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA GCCCAGCTTG  
8941 GAGCGAACGA CCTACCCGA ACTGAGATAC CTACAGCGTG AGCATTGAGA AAGCGCCACG  
9001 CTTCCCGAAG GGAGAAAGGC GGAGAGGTAT CCGGTAAGCG GCAGGGTCCG AACAGGAGAG  
9061 CGCACGAGGG AGCTTCCAGG GGGGAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTTCG  
9121 CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCCGAG CCTATGGAAA  
9181 AACGCCAGCA ACGCGGCCCT TTTACGTTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG  
9241 TTCTTTCTCT CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT  
9301 GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA  
9361 GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC CGATTCATTA ATGCAGCTGG-

FIGURE 41D

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9421 CACGACAGGT TTCCCGACTG GAAAGCGGGC AGTGAGCGCA ACGCAATTAA TGTGAGTTAC  
9481 CTCACTCATT AGGCACCCCA GGCTTTACAC TTTATGCTTC CGGCTCCTAT GTTGTGTGGA  
9541 ATTGTGAGCG GATAACAATT TCACACAGGA AACAGCTATG ACCATGATT A CGCCAAGCTC  
9601 GGAATTAACC CTCACTAAAG GGAACAAAAG CTGGTACCGA TCCCGAGCTT TGCAAATTAA  
9661 AGCCTTCGAG CGTCCCAAAA CCTTCTCAAG CAAGGTTTTT AGTATAATGT TACATGCGTA  
9721 CACGCGTCTG TACAGAAAAA AAAGAAAAAT TTGAAATATA AATAACGTTT TTAATACTAA  
9781 CATAACTATA AAAAAATAAA TAGGGACCTA GACTTCAGGT TGTCTAACTC CTTCCTTTTT  
9841 GGTTAGAGCG GATGTGGGGG GAGGGCGTGA ATGTAAGCGT GACATAACTA ATTACATGAT  
9901 ATCGACAAAG GAAAAGGGGC CTGTTTACTC ACAGGCTTTT TTCAAGTAGG TAATTAAGTC  
9961 GTTCTGTCT TTTTCTTCT TCAACCCACC AAAGGCCATC TTGGTACTTT TTTTTTTTTT  
10021 TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT  
10081 TTTTTTTTTT TTTTTTTTTT TCATAGAAAT AATACAGAAG TAGATGTTGA ATTAGATTAA  
10141 ACTGAAGATA TATAATTTAT TGGAAAATAC ATAGAGCTTT TTGTTGATGC GCTTAAGCGA  
10201 TCAATTCAAC AACACCACCA GCAGCTCTGA TTTTTTCTTC AGCCAACCTG GAGACGAATC  
10261 TAGCTTTGAC GATAACTGGA ACATTTGGAA TTCTACCCTT ACCCAAGATC TTACCGTTAA  
10321 CGGCTGCCAA AGTGTCAATA ACTGGAGCAG TTTCTTAGA AGCAGATTTC AAGTATTGGT  
10381 CTCTCTGTG TCTGCGGATC AATGTCCACA ATTTGTCCAA GTTCAAGACT GGCTTCCAGA  
10441 AATGAGCTTG TTGCTTGTGG AAGTATCTCA TACCAACCTT ACCGAAATAA CCTGGATGGT  
10501 ATTTATCCAT GTTAATTCTG TGGTGTGTT GACCACCGGC CATACCTCTA CCACCGGGGT  
10561 GCTTCTGTG CTTACCGATA CGACCTTTAC CGGCTGAGAC GTGACCTCTG TGCTTTCTAG  
10621 TCTTAGTGAA TCTGGAAGGC ATTCTTGATT AGTTGGATGA TTGTTCTGGG ATTTAATGCA  
10681 AAAATCACTT AAGAAGGAAA ATCAACGGAG AAAGCAAACG CCATCTTAAA TATACGGGAT  
10741 ACAGATGAAA GGGTTTGAAC CTATCTGGAA AATAGCATT AACAAGCGAA AAAGTCCGAG  
10801 GAAAATTGTT TCGCTCTCTG CGGGCTATTC ACGCGCCAGA GGAAAATAGG AAAAATAACA  
10861 GGGCATTAGA AAAATAATTT TGATTTTGGT AATGTGTGGG TCCTGGTGTA CAGATGTTAC  
10921 ATTGGTTACA GTACTCTTGT TTTTGTGTG TTTTTCGATG AATCTCCAAA ATGGTTGTTA  
10981 GCACATGGAA GAGTCACCGA TGCTAAGTTA TCTCTATGTA AGCTACGTGG CGTGACTTTT  
11041 GATGAAGCCG CACAAGAGAT ACAGGATTGG CAACTGCAAA TAGAATCTGG GGATCCCCC  
11101 TCGAGATCCG GGATCGAAGA AATGATGGTA AATGAAATAG GAAATCAAGG AGCATGAAGG  
11161 CAAAAGACAA ATATAAGGGT CGAACGAAAA ATAAAGTGAA AAGTGTGAT ATGATGTATT  
11221 TGGCTTTGCG GCGCCGAAAA AACGAGTTTA CGCAATTGCA CAATCATGCT GACTCTGTGG  
11281 CGGACCCGCG CTCTTGCCGG CCCGGCGATA ACGCTGGGCG TGAGGCTGTG CCCGGCGGAG  
11341 TTTTTTGCGC CTGCATTTTC CAAGGTTTAC CCTGCGCTAA GGGGCGAGAT TGGAGAAGCA  
11401 ATAAGAATGC CGGTTGGGGT TGCGATGATG ACGACCACGA CAACTGGTGT CATTATTTAA  
11461 GTTGCCGAAA GAACCTGAGT GCATTTGCAA CATGAGTATA CTAGAAGAAT GAGCCAAGAC  
11521 TTGCGAGACG CGAGTTTGCC GGTGGTGCGA ACAATAGAGC GACCATGACC TTGAAGGTGA  
11581 GACGCGCATA ACCGCTAGAG TACTTTGAAG AGGAAACAGC AATAGGGTTG CTACCAGTAT  
11641 AAATAGACAG GTACATACAA CACTGGAAAT GGTTGTCTGT TTGAGTACGC TTTCAATTCA  
11701 TTTGGGTGTG CAC

FIGURE 415

Figure 42A:

pDEST22

2-Hybrid Vector with  
Activation Domain

657 acg cac act act ctc taa tga gca acg gta tac ggc ctt cct tcc agt tac  
tgc gtg tga tga gag att act cgt tgc cat atg ccg gaa gga agg tca atg

708 ttg aat ttg aaa taa aaa aag ttt gcc gct ttg cta tca agt ata aat aga  
aac tta aac ttt att ttt ttc aaa cgg cga aac gat agt tca tat tta tct

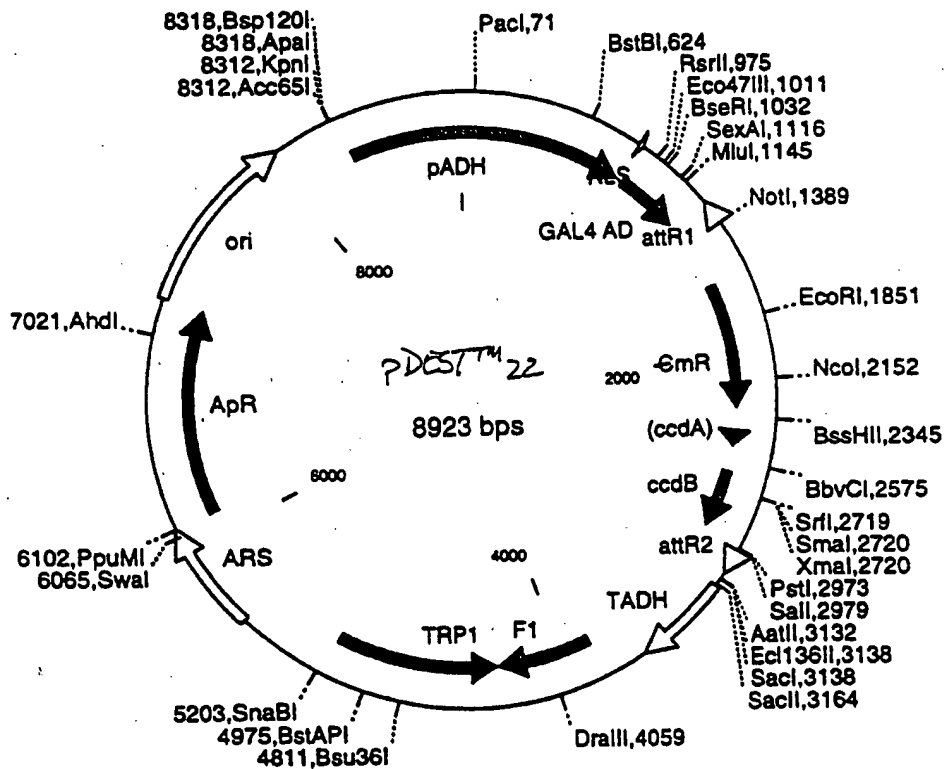
759 cct gca att att aat ctt ttg ttt cct cgt cat tgt tct cgt tcc ctt tct  
gga cgt taa taa tta gaa aac aaa gga gca gta aca aga gca agg gaa aga

810 *ADH Promoter*  
"ccc/ttg ttt ttt ttt ctc cat aat att tca agc tat acc aag cat aca atc"  
"agg aac aaa gaa aaa gac gtg tta taa agt tgc ata tgg ttc gta tct tag"

861 *GAL4-AD*  
"aac/ttc aag ctt atg ccc aag aag aag cgg aag gtc tgc agc ggc gcc aat"  
"ctg agg ttc gaa tac ggg ttc ttc ttc gcc ttc cag agc tgc ccg cgg ttc"  
*Start Translation*

1218 gaa gat acc cca cca aac cca aaa aaa gag ggt ggg tgc aat caa *D G G S N Q T S* aca agt  
ctt cta tgg ggt ggt ttg ggt ttt ttt ctc cca ccc agc tta gtt tgt tca

1269 *LYKKAA*  
"ttg tac aaa aaa gct gaa cga gaa acg taa a"  
"aac atg ttt ttt cga ctt gct ctt tgc att t"  
*Intv*





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## pDEST22 8923 bp

Location (Base Nos.)	Gene Encoded
904..1248	GAL4 AD
1388..1264	attR1
1638..2297	CmR
2417..2501	inactivated ccdA
2639..2944	ccdB
2985..3109	attR2
3831..4318	f1 (f1 intergenic region)
4334..5176	TRP1
6110..7194	ampR
8344..866	pADH (yeast ADH promoter)

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1  TTCATTGGG TGTGCACTTT ATTATGTTAC AATATGGAAG GGAACTTTAC ACTTCTCCTA
61  TGCACATATA TTAATTAAAG TCCAATGCTA GTAGAGAAGG GGGGTAACAC CCTCCGCGC
121 TCTTTTCCGA TTTTCTCTA AACCGTGGAA TATTTCCGGAT ATCCTTTTGT TGTTCGCGG
181 TGTACAATAT GGACTTCCTC TTTTCTGGCA ACCAAACCCA TACATCGGGA TTCCTATAAT
241 ACCTTCGTTG GTCTCCCTAA CATGTAGGTG GCGGAGGGGA GATATACAAT AGAACAGATA
301 CCAGACAAGA CATAATGGGC TAAACAAGAC TACACCAATT ACACCTGCCTC ATTGATGGTG
361 GTACATAACG AACTAATACT GTAGCCCTAG ACTTGATAGC CATCATCATA TCGAAGTTTC
421 ACTACCCCTT TTCCATTGTC CATCTATTGA AGTAATAATA GGCGCATGCA ACTTCTTTTC
481 TTTTCTTTTC TTTTCTCTCT CCCCCGTGT TGTCTCACCA TATCCGCAAT GACAAAAAAA
541 ATGATGGAAG AACTAAAGG AAAAAATTAA CGACAAAGAC AGCACCAACA GATGTCGTTG
601 TTCCAGAGCT GATGAGGGGT ATCTTCGAAC ACACGAAACT TTTTCTTCC TTCATTACG
661 CACACTACTC TCTAATGAGC AACGGTATAC GGCCTTCCTT CCAGTTACTT GAATTTGAAA
721 TAAAAAAGT TTGCCGCTTT GCTATCAAGT ATAAATAGAC CTGCAATTAT TAATCTTTTG
781 TTTCTCTGTC ATTGTTCTCG TTCCCTTTCT TCCTTGTTTC TTTTCTGCA CAATATTTCA
841 AGCTATACCA AGCATACAAT CAACTCCAAG CTTATGCCCA AGAAGAAGCG GAAGGTCCTG
901 AGCGGCGCCA ATTTAATCA AAGTGGGAAT ATTGCTGATA GCTCATTGTC CTTCACTTTT
961 ACTAACAGTA GCAACGGTCC GAACCTCATA ACAACTCAA CAAATTCTCA AGCGCTTTCA
1021 CAACCAATTG CCTCCTCTAA CGTTCATGAT AACTTCATGA ATAATGAAAT CACGGCTAGT
1081 AAAATTGATG ATGGTAATAA TTCAAAACCA CTGTACCTG GTTGGACGGA CCAAACTGCG
1141 TATAACGCGT TTGGAATCAC TACAGGGATG TTTAATACCA CTACAATGGA TGATGTATAT
1201 AACTATCTAT TCGATGATGA AGATACCCCA CCAAACCCAA AAAAAGAGGG TGGGTGCAAT
1261 CAAACAAGTT TGTACAAAAA AGCTGAACGA GAAACGTAAA ATGATATAAA TATCAATATA
1321 TTAAATTAGA TTTTGCATAA AAAACAGACT ACATAATACT GTAAAAACACA ACATATCCAG
1381 TCACTATGGC GGCCGCTAAG TTGGCAGCAT CACCCGACGC ACTTTGCGCC GAATAAATAC
1441 CTGTGACGGA AGATCACTTC GCAGAATAAA TAAATCCTGG TGTCCCTGTT GATACCGGGA
1501 AGCCCTGGGC CAACTTTTGG CGAAAATGAG ACGTTGATCG GCACGTAAGA GGTTCCTCACT
1561 TTCACCATAA TGAAATAAGA TCACTACCGG GCGTATTTTT TGAGTTATCG AGATTTTCAG
1621 GAGCTAAGGA AGCTAAATG GAGAAAAAAA TCACTGGATA TACCACCGTT GATATATCCC
1681 AATGGCATCG TAAAGAACAT TTTGAGGCAT TTCAGTCAGT TGCTCAATGT ACCTATAACC
1741 AGACCGTTCA GCTGGATATT ACGGCCTTTT TAAAGACCGT AAAGAAAAAT AAGCACAAAT
1801 TTTATCCGGC CTTTATTCAC ATTCTTGCCC GCCTGATGAA TGCTCATCCG GAATTCCGTA
1861 TGGCAATGAA AGACGGTGAG CTGGTGATAT GGGATAGTGT TCACCCTTGT TACACCGTTT
1921 TCCATGAGCA AACTGAAACG TTTTCATCGC TCTGGAGTGA ATACCACGAC GATTTCCGGC
1981 AGTTTCTACA CATATATTCG CAAGATGTGG CGTGTACGG TGAAAACCTG GCCTATTTCC
2041 CTAAGGGT TATTGAGAAT ATGTTTTTCG TCTCAGCCAA TCCTTGGGTG AGTTTCACCA
2101 GTTTTGATTT AAACGTGGCC AATATGGACA ACTTCTTCGC CCCCCTTTTC ACCATGGGCA
2161 AATATTATAC GCAAGGCGAC AAGGTGCTGA TGCCGCTGGC GATTCAAGTT CATCATGCCG
2221 TCTGTGATGG CTTCCATGTC GGCAGAATGC TTAATGAATT ACAACAGTAC TCGCATGAGT
2281 GGCAGGCGG GCGTAATCT AGAGGATCCG GCTTACTAAA AGCCAGATAA CAGTATGCGT
2341 ATTTGCGCGC TGATTTTTCG GGTATAAGAA TATATACTGA TATGTATACC CGAAGTATGT
2401 CAAAAAGAGG TGTGCTATGA AGCAGCGTAT TACAGTGACA GTTGACAGCG ACAGCTATCA
2461 GTTGCTCAAG GCATATATGA TGTCAATATC TCCGGTCTGG TAAGCACAAC CATGCAGAA
2521 GAAGCCCGTC GTCTGCGTGC CGAACGCTGG AAAGCGGAAA ATCAGGAAGG GATGGCTGAG-

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FIGURE 425

2581 GTCGCCCGGT TTATTGAAAT GAACGGCTCT TTTGCTGACG AGAACAGGGA CTGGTGAAAT  
2641 GCAGTTTAAG GTTACACCT ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA  
2701 GAGTGATATT ATTGACACGC CCGGGCGACG GATGGTGATC CCCCTGGCCA GTGCACGTCT  
2761 GCTGTCAGAT AAAGTCTCCC GTGAACTTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG  
2821 GCGCATGATG ACCACCGATA TGGCCAGTGT GCCGCTCTCC GTTATCGGGG AAGAAGTGGC  
2881 TGATCTCAGC CACCGCGAAA ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT  
2941 ATAAATGTCA GGCTCCCTTA TACACAGCCA GTCTGCAGGT CGACCATAGT GACTGGATAT  
3001 GTTGTGTTTT ACAGTATTAT GTAGTCTGTT TTTTATGCAA AATCTAATTT AATATATTGA  
3061 TATTTATATC ATTTTACGTT TCTCGTTTCT CTTTCTTGTA CAAAGTGGTT TGATGGCCGC  
3121 TAAGTAAGTA AGACGTCGAG CTCTAAGTAA GTAACGCCCG CCACCGCGGT GGAGCTTTGG  
3181 ACTTCTTCGC CAGAGGTTTG GTCAAGTCTC CAATCAAGGT TGTCGGCTTG TCTACCTTGC  
3241 CAGAAATTTA CGAAAAGATG GAAAAGGGTC AAATCGTTGG TAGATACGTT GTTGACACTT  
3301 CTAAATAAGC GAATTTCTTA TGATTTATGA TTTTATTAT TAAATAAGTT ATAAAAAATA  
3361 TAAGTGATA CAAATTTTAA AGTGAATCTT AGGTTTTAAA ACGAAAATTC TTATTCTTGA  
3421 GTAATCTTTT CCTGTAGGTC AGGTTGCTTT CTCAGGTATA GCATGAGGTC GCTCTTATTG  
3481 ACCACACCTC TACCGGCATG CCGAGCAAAT GCCTGCAAAT CGCTCCCAT TTACCCCAAT  
3541 TGTAGATATG CTAACCTCCAG CAATGAGTTG ATGAATCTCG GTGTGTATTT TATGTCTCA  
3601 GAGGACAATA CCTGTTGTAA TCGTTCTTCC ACACGGATCC CAATTCGCCC TATAGTGAGT  
3661 CGTATTACAA TTCCTGGCC GTCGTTTTAC AACGTCGTGA CTGGGAAAAC CCTGGCGTTA  
3721 CCCAATTTAA TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCGTAAT AGCGAAGAGG  
3781 CCCGCACCGA TCGCCTTCC CAACAGTTGC GCAGCCTGAA TGGCGAATGG ACGCGCCCTG  
3841 TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTACGCGC AGCGTGACCG CTACACTTGC  
3901 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTGCGCA CGTTCGCCGG  
3961 CTTTCCCGGT CAAGCTCTAA ATCGGGGGCT CTTTTAGGG TTCCGATTTA GTGCTTTACG  
4021 GCACCTCGAC CCCAAAAAAC TTGATTAGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG  
4081 ATAGACGGTT TTTGCGCCTT TGACGTTGGA GTCCACGTTT TTTAATAGTG GACTCTTGTT  
4141 CCAAACTGGA ACAACACTCA ACCCTATCTC GGTCTATTCT TTTGATTAT AAGGGATTTT  
4201 GCCGATTTTC GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT  
4261 TAACAAAATA TTAACGTTTA CAATTTCTCTG ATGCGGTATT TTCTCCTTAC GCATCTGTGC  
4321 GGTATTTTCA ACCGCAGGCA AGTGACAAA CAATACTTAA ATAAATACTA CTCAGTAATA  
4381 ACCTATTTCT TAGCATTTT GACGAAATTT GCTATTTTGT TAGAGTCTTT TAGACATTT  
4441 GTCCTCCAC CTTCCGCTTAC ATCAACACCA ATAACGCCAT TTAATCTAAG CGCATCACCA  
4501 ACATTTTCTG GCGTCAGTCC ACCAGCTAAC ATAAATGTAA AGCTTTCGGG GCTCTCTTGC  
4561 CTTCCAACCC AGTCAGAAAT CGAGTTCCAA TCCAAAAGTT CACCTGTCCC ACCTGCTTCT  
4621 GAATCAAACA AGGGAATAAA CGAATGAGGT TTCTGTGAAG CTGCACTGAG TAGTATGTTG  
4681 CAGTCTTTTG GAAATACGAG TCTTTTAATA ACTGGCAAAC CGAGGAACTC TTGGTATTCT  
4741 TGCCACGACT CATCTCCATG CAGTTGGACG ATATCAATGC CGTAATCATT GACCAGAGCC  
4801 AAAACATCCT CTTAGGTTG ATTACGAAAC ACGCCAACCA AGTATTTGCG AGTGCCCTGAA  
4861 CTATTTTTAT ATGCTTTTAC AAGACTTGAA ATTTTCTTG CAATAACCGG GTCAATTGTT  
4921 CTCTTTCTAT TGGGCACACA TATAATACCC AGCAAGTCAG CATCGGAATC TAGAGCACAT  
4981 TCTGCGGCT CTGTGCTCTG CAAGCCGCAA ACTTTCACCA ATGGACCAGA ACTACCTGTG  
5041 AAATTAATAA CAGACATACT CCAAGCTGCC TTTGTGTGCT TAATCACGTA TACTCACGTG  
5101 CTCAATAGTC ACCAATGCCC TCCCTCTTGG CCTCTCTCTT TTCTTTTTTC GACCGAATTA  
5161 ATTCTTAATC GGCAAAAAA GAAAAGCTCC GGATCAAGAT TGTACGTAAG GTGACAAGCT  
5221 ATTTTTCAT AAAGAATATC TTCCACTACT GCCATCTGGC GTCATACTG CAAAGTACAC  
5281 ATATATTACG ATGCTGTCTA TTAATGCTT CCTATATTAT ATATATAGTA ATGTCGTTTA  
5341 TGGTGCACTC TCAGTACAAT CTGCTCTGAT GCCGCATAGT TAAGCCAGCC CCGACACCCG  
5401 CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA  
5461 GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC  
5521 GCGAGACGAA AGGGCCTCGT GATACGCTTA TTTTATAGG TTAATGTCAT GATAATAATG  
5581 GTTCTTAGG ACGGATCGCT TGCTGTAAAC TTACACGCGC CTCGTATCTT TTAATGATGG  
5641 AATAATTTGG GAATTTACTC TGTGTTTATT TATTTTATG TTTTGTATTT GGATTTTAGA  
5701 AAGTAAATAA AGAAGGTAGA AGAGTTACGG AATGAAGAAA AAAAAATAA CAAAGGTTTA  
5761 AAAAAATTTA AAAAAAGCG TACTTTACAT ATATATTTAT TAGACAAGAA AAGCAGATTA  
5821 AATAGATATA CATTGATTA AAGTGAATAA TCACAGGATT TTCGTGTGTG  
5881 GTCTTCTACA CAGACAAGAT GAAACAATTC GGCATTAATA CCTGAGAGCA GGAAGAGCAA  
5941 GATAAAAGGT AGTATTTGTT GGCATCCCC CTAGAGTCTT TTACATCTC GGAACACAA  
6001 AACTATTTTT TCTTTAATTT CTTTTTTTAC TTTCTATTTT TAATTTATAT ATTTATATTA-

FIGURE 42c

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6061 AAAAATTTAA ATTATAATTA TTTTATAGC ACGTGATGAA AAGGACCCAG GTGGCACTTT  
6121 TCGGGGAAAT GTGCGCGGAA CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA  
6181 TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT  
6241 GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT  
6301 TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG  
6361 AGTGGGTTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA  
6421 AGAACGTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG  
6481 TATTGACGCC GGGCAAGAGC AACTCGGTG CCGCATACAC TATTCTCAGA ATGACTTGTT  
6541 TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG  
6601 CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG  
6661 AGGACCGAAG GAGCTAACCG CTTTTTTTCA CAACATGGGG GATCATGTAA CTCGCCTTGA  
6721 TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC  
6781 TGTAGCAATG GCAACAACGT TCGGCAAACT ATTAAGTGGC GAAGTACTTA CTCTAGCTTC  
6841 CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC  
6901 GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG  
6961 CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC  
7021 GACGGGCAGT CAGGCACTA TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC  
7081 ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT  
7141 AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC  
7201 CAAAATCCCT TAACGTGAGT TTTCTGTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA  
7261 AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAA CAAAAAACC  
7321 ACCGCTACCA GCGGTGTTTT GTTTCGCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT  
7381 AACTGGCTTC AGCAGAGCGC AGATACCAA TACTGTCTTT CTAGTGTAGC CGTAGTTAGG  
7441 CCACCACTTC AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC  
7501 AGTGGCTGCT GCCAGTGGCG ATAAGTCTGT TCTTACCGGG TTGGACTCAA GACGATAGTT  
7561 ACCGGATAAG GCGCAGCGGT CCGGCTGAAC GGGGGGTTCC TGACACAGC CCAGCTTGGA  
7621 GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT  
7681 TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG  
7741 CACGAGGGAG CTTCCAGGGG GGAACGCCCTG GTATCTTTAT AGTCCTGTCT GGTTCGCCA  
7801 CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCCGAGCC TATGGAAAAA  
7861 CGCCAGCAAC GCGGCCTTTT TACGGTTTCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT  
7921 CTTTCCTGCG TTATCCCCTG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA  
7981 TACCGCTCGC CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA  
8041 GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA  
8101 CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTACCT  
8161 CACTCATTAG GCACCCCAAG CTTTACACTT TATGCTTCCG GCTCCTATGT TGTGTGGAAT  
8221 TGTGAGCGGA TAACAATTTT ACACAGGAAA CAGCTATGAC CATGATTACG CCAAGCTCGG  
8281 AATTAACCTT CACTAAAGGG AAAAAAGCT GGGTACCGGG CCCCCCTCG AGATCCGGGA  
8341 TCGAAGAAAT GATGGTAAAT GAAATAGGAA ATCAAGGAGC ATGAAGGCAA AAGACAAATA  
8401 TAAGGGTCGA ACGAAAAATA AAGTGAAGA TGTGATATG ATGTATTTGG CTTTGCGGCG  
8461 CCGAAAAAAC GAGTTTACGC AATTGCACAA TCATGCTGAC TCTGTGGCGG ACCCGCGCTC  
8521 TTGCCGGCCC GCGGATAACG CTGGGCGTGA GGCTGTGCCC GCGCGAGTTT TTGCGCCTG  
8581 CATTTTCCAA GGTTTACCCT GCGCTAAGGG GCGAGATTGG AGAAGCAATA AGAATGCCGG  
8641 TTGGGGTTGC GATGATGACG ACCACGACAA CTGGTGTCAT TATTTAAGTT GCCGAAAGAA  
8701 CCTGAGTGCA TTTGCAACAT GAGTATACTA GAAGAATGAG CCAAGACTTG CGAGACGCGA  
8761 GTTTGCCGGT GGTGCGAACA ATAGAGCGAC CATGACCTTG AAGGTGAGAC GCGCATAACC  
8821 GCTAGAGTAC TTTGAAGAGG AAACAGCAAT AGGGTTGCTA CCAGTATAAA TAGACAGGTA  
8881 CATACAACAC TGGAAATGGT TGTCTGTTTG AGTACGCTTT CAA

FIGURE 42A



## pDEST23 6264 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
285..161		attR1
394..1053		CmR
1173..1257		inactivated ccdA
1395..1700		ccdB
1741..1865		attR2
1883..1911		his6
2574..3434		ampR
3583..4222		ori
1	TCTTCCCCAT CGGTGATGTC GCGCATATAG GCGCCAGCAA CCGCACCTGT GGC GCCGGTG	
61	ATGCCGGCCA CGATGCGTCC GCGTAGAGG ATCGAGATCT CGATCCCGCG AAATTAATAC	
121	GACTCACTAT AGGGAGACCA CAACGGTTTC CCTCTAGATC ACAAGTTTGT ACAAAAAAGC	
181	TGAACGAGAA ACGTAAATG ATATAAATAT CAATATATTA AATTAGATTT TGCATAAAAA	
241	ACAGACTACA TAATACTGTA AAACACAACA TATCCAGTCA CTATGGCGGC CGCATTAGGC	
301	ACCCAGGCT TTACACTTTA TGCTCCGGC TCGTATAATG TGTGGATTTT GAGTTAGGAT	
361	CCGGCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGGAGA AAAAAATCAC TGGATATACC	
421	ACCGTTGATA TATCCCAATG GCATCGTAAA GAACATTTTG AGGCATTTCA GTCAGTTGCT	
481	CAATGTACCT ATAACCAGAC CGTTCAGCTG GATATTACGG CCTTTTAAA GACCGTAAAG	
541	AAAAATAAGC ACAAGTTTTA TCCGGCCTTT ATTCACATTC TTGCCCGCCT GATGAATGCT	
601	CATCCGGAAT TCCGTATGGC AATGAAAGAC GGTGAGCTGG TGATATGGGA TAGTGTTCAC	
661	CCTTGTTACA CCGTTTTCCA TGAGCAAAC GAAACGTTTT CATCGCTCTG GAGTGAATAC	
721	CACGACGATT TCCGGCAGTT TCTACACATA TATTCGCAAG ATGTGGCGTG TTACGGTGAA	
781	AACCTGGCCT ATTTCCCTAA AGGGTTTATT GAGAATATGT TTTTCGTCTC AGCCAATCCC	
841	TGGGTGAGTT TCACCAAGTT TGATTTAAAC GTGGCCAATA TGGACAACCT CTTCGCCCCC	
901	GTTTTACCA TGGGCAAATA TTATACGCAA GCGGACAAGG TGCTGATGCC GCTGGCGATT	
961	CAGGTTTCAT ATGCCGCTG TGATGGCTG CATGTCGGCA GAATGCTTAA GATATTACAA	
1021	CAGTACTGCG ATGAGTGCA GGGCGGGGCG TAAACGCGTG GATCCGGCTT ACTAAAAGCC	
1081	AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGGGTA TAAGAATATA TACTGATATG	
1141	TATACCCGAA GTATGTCAA AAGAGGTGTG CTATGAAGCA GCGTATTACA GTGACAGTTG	
1201	ACAGCGACAG CTATCAGTTG CTCAGGCGAT ATATGATGTC AATATCTCCG GTCTGGTAAAG	
1261	CACAACCATG CAGAATGAAG CCCGTCGTCT GCGTGCCGAA CGCTGGAAAG CGGAAATCA	
1321	GGAAGGGATG GCTGAGGTCG CCCGGTTTAT TGAAATGAAC GGCTCTTTTG CTGACGAGAA	
1381	CAGGGAGCTG TGAAATGCAG TTAAAGGTTT ACACCTATAA AAGAGAGAGC CGTTATCGTC	
1441	TGTTTGTGGA TGTACAGAGT GATATTATTG ACACGCCCGG GCGACGGATG GTGATCCCCC	
1501	TGGCCAGTGC ACGTCTGCTG TCAGATAAAG TCTCCCGTGA ACTTTACCCG GTGGTGCATA	
1561	TCGGGGATGA AAGCTGGCGC ATGATGACCA CCGATATGGC CAGTGTGCCG GTCTCCGTTA	
1621	TCGGGGAAGA AGTGGCTGAT CTCAGCCACC GCGAAAATGA CATCAAAAAC GCCATTAACC	
1681	TGATGTTCTG GGGAAATATA ATGTCAGGCT CCCTTATACA CAGCCAGTCT GCAGGTCGAC	
1741	CATAGTGAAT GGATATGTTG TGTTTTACAG TATTATGTAG TCTGTTTTTT ATGCAAAATC	
1801	TAATTTAATA TATTGATATT TATATCATTT TACGTTTCTC GTTCAGCTTT CTTGTACAAA	
1861	GTGGTGATTA TGTCGTACTA CCATCACCAT CACCATCACC TCGATGAGCA ATAACCTAGCA	
1921	TAACCCCTTG GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGCTGAAAGG AGGAACCTATA	
1981	TCCGGATATC CACAGGACGG GTGTGGTCGC CATGATCGCG TAGTCGATAG TGGCTCCAAG	
2041	TAGCGAAGCG AGCAGGACTG GCGGCGGCC AAAGCGGTCG GACAGTGCTC CGAGAACGGG	
2101	TGCGCATAGA AATTGCATCA ACGCATATAG CGCTAGCAGC ACGCCATAGT GACTGGCGAT	
2161	GCTGTCGGAA TGGACGATAT CCCGCAAGAG GCCCGGCAGT ACCGGCATAA CCAAGCCTAT	
2221	GCCTACAGCA TCCAGGGTGA CGGTGCCGAG GATGACGATG AGCGCATTGT TAGATTTCAT	
2281	ACACGGTGCC TGACTGCGTT AGCAATTTAA CTGTGATAAA CTACCGCATT AAAGCCTATC	
2341	GATGATAAGC TGTCAAACAT GAGAATTCTT GAAGAAGAAA GGGCCTCGTG ATACGCCCTAT	
2401	TTTATAGGT TAATGTCATG ATAATAATGG TTTCTTAGAC GTCAGGTGGC ACTTTTCGGG	
2461	GAAATGTGCG CGGAACCCCT ATTTGTTTAT TTTTCTAAAT ACATTCAAAT ATGTATCCGC	
2521	TCATGAGACA ATAACCCTGA TAAATGCTTC AATAATATTG AAAAAGGAAG AGTATGAGTA	
2581	TTCAACATTT CCGTGTGCGC CTTATTCCCT TTTTTCGGGC ATTTTGCCTT CCTGTTTTTG	
2641	CTCACCCAGA AACGCTGGTG AAAGTAAAG ATGCTGAAGA TCAGTTGGGT GCACGAGTGG	

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2701 GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTTTCGC CCCGAAGAAC  
2761 GTTTTCCAAT GATGAGCACT TTTAAAGTTC TGCTATGTGG CCGGGTATTA TCCCGTGTG  
2821 ACGCCGGGCA AGAGCAACTC GGTGCGCCGA TACACTATTC TCAGAATGAC TTGGTTGAGT  
2881 ACTCACCAGT CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG  
2941 CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC  
3001 CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT  
3061 GGGAAACCGGA GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGCAG  
3121 CAATGGCAAC AACGTTGCGC AAACATTTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC  
3181 AACAAATTAAT AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC  
3241 TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG TCTCGCGGTA  
3301 TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG  
3361 GGATGCAGG AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA  
3421 TTAAGCATTG GTAACGTGCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAC  
3481 TTCATTTTTTA ATTTAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA  
3541 TCCCTTAACG TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT  
3601 CTTCTTGAGA TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC  
3661 TACCAGCGGT GGTGTTGTTG CCGGATCAAG AGCTACCAAC TCTTTTTCCG AAGGTAACGT  
3721 GCTTCAGCAG AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC  
3781 ACTTCAAGAA CTCTGTAGCA CCGCTACAT ACCTCGCTCT GCTAATCCTG TTACCACTGG  
3841 CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG  
3901 ATAAGGCGCA GCGGTGCGGC TGAACGGGGG GTTCGTGCAC ACAGCCGAGC TTGGAGCGAA  
3961 CGACCTACAC CGAACTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG  
4021 AAGGGAGAAA GCGGACAGG TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA  
4081 GGGAGCTTCC AGGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT  
4141 GACTTGAGCG TCGATTTTTG TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAACGCCA  
4201 GCAACGCGGC CTTTTTACGG TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC ATGTTCTTTC  
4261 CTGCGTTATC CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG  
4321 CTCGCCGCG CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC  
4381 TGATGCGGTA TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA TATGGTGCAC  
4441 TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACACTCC GCTATCGCTA  
4501 CGTGACTGGG TCATGGCTGC GCCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG  
4561 GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG  
4621 TGTCAGAGGT TTTACCGTC ATCACCAGAA CGCGCGAGGC AGCTGCGGTA AAGCTCATCA  
4681 GCGTGGTCGT GAAGCGATT ACAGATGTCT GCCTGTTCTT CCGCGTCCAG CTCGTTGAGT  
4741 TTCTCCAGAA GCGTTAATGT TTGGCTTCTG ATAAAGCGGG CCATGTTAAG GCGGGTTTTT  
4801 TCCTGTTTTG TCACTGATGC CTCGGTGTA GGGGGATTTT TGTTTCATGGG GGTAAATGATA  
4861 CCGATGAAAC GAGAGAGGAT GCTCACGATA CCGGTTACTG ATGATGAACA TGCCCGGTTA  
4921 CTGGAACGTT GTGAGGGTAA ACAACTGGCG GTATGGATGC GCGGGGACCA GAGAAAAATC  
4981 ACTCAGGGTC AATGCCAGCG CTTCTGTTAAT ACAGATGTAG GTGTTCCACA GGGTAGCCAG  
5041 CAGCATCCTG CGATGCAGAT CCGGAACATA ATGGTGCAGG GCGCTGACTT CCGCGTTTCC  
5101 AGACTTTACG AAACACGGAA ACCGAAGACC ATTCATGTTG TTGCTCAGGT CGCAGACGTT  
5161 TTGCAGCAGC AGTCGCTTCA CGTTCGCTCG CGTATCGGTG ATTCATTCTG CTAACCAAGTA  
5221 AGGCAACCCC GCCAGCCTAG CCGGTCCTC AACGACAGGA GCACGATCAT GCGCACCCGT  
5281 GGCCAGGACC CAACGCTGCC CGAGATGCGC CGCGTGCGGC TGCTGGAGAT GCGGACGCG  
5341 ATGGATATGT TCTGCCAAGG GTTGGTTTGC GCATTCACAG TTCTCCGCAA GAATTGATTG  
5401 GCTCCAATTC TTGGAGTGGT GAATCCGTTA GCGAGGTGCC GCGGGCTTCC ATTCAGGTG  
5461 AGGTGGCCCG GCTCCATGCA CCGCGACGCA ACGCGGGGAG GCAGACAAGG TATAGGGCGG  
5521 CGCCTACAAT CCATGCCAAC CCGTTCCATG TGCTCGCCGA GCGCGCATAA ATCGCGGTGA  
5581 CGATCAGCGG TCCAGTGATC GAAGTTAGGC TGGTAAGAGC CGCGAGCGAT CCTTGAAGCT  
5641 GTCCCTGATG GTCGTCACT ACCTGCCTGG ACAGCATGGC CTGCAACGCG GGCATCCCCG  
5701 TGCCGCCGGA AGCGAGAAGA ATCATAATGG GGAAGGCCAT CCAGCTCGC GTCGCGAACG  
5761 CCAGCAAGAC GTAGCCCAGC GCGTCGGCGG CCATGCCGCG GATAATGGCC TGCTTCTCGC  
5821 CGAAACGTTT GGTGGCGGGA CCAGTGACGA AGGCTTGAGC GAGGGCGTGC AAGATTCCGA  
5881 ATACCGCAAG CGACAGGCCG ATCATCGTCG CGCTCCAGCG AAAGCGGTCC TCGCCGAAAA  
5941 TGACCCAGAG CGCTGCCGGC ACCTGTCTTA CGAGTTGCAT GATAAAGAAG ACAGTCATAA  
6001 GTGCGGCGAC GATAGTCATG CCCCAGCGCC ACCGGAAGGA GCTGACTGGG TTGAAGGCTC  
6061 TCAAGGGCAT CGGTCGATCG ACGCTCTCCC TTATGCGACT CCTGCATTAG GAAGCAGCCC  
6121 AGTAGTAGGT TGAGGCCGTT GAGCACCGCC GCCGCAAGGA ATGGTGCAAT CAAGGAGATG -

FIGURE 43C

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6181 GCGCCCAACA GTCCCCCGGC CACGGGGCCT GCCACCATAC CCACGCCGAA ACAAGCGCTC  
6241 ATGAGCCCGA AGTGGCGAGC CCGA

FIGURE 43D

PD EST 24

11 ————— cmR ————— ccdB ————— 11

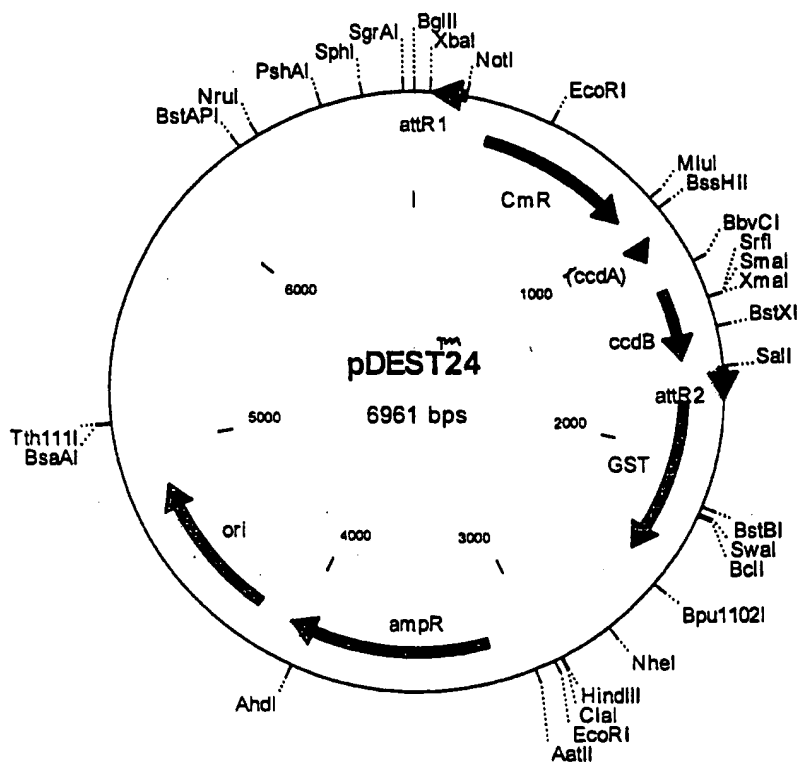


FIGURE 44A



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## pDEST24 6961 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
195..71		attR1
304..963		CmR
1083..1167		inactivated ccdA
1305..1610		ccdB
1651..1775		attR2
1783..2451		GST
3181..4041		ampR
4190..4829		ori

1	ATCGAGATCT	CGATCCCGCG	AAATTAATAC	GACTCACTAT	AGGGAGACCA	CAACGGTTTC
61	CCTCTAGATC	ACAAGTTTGT	ACAAAAAAGC	TGAACGAGAA	ACGTAAAATG	ATATAAATAT
121	CAATATATTA	AATTAGATTT	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA
181	TATCCAGTCA	CTATGGCGGC	CGCATTAGGC	ACCCCAGGCT	TTACACTTTA	TGCTTCCGGC
241	TCGTATAATG	TGTGGATTTT	GAGTTAGGAT	CCGGCGAGAT	TTTCAGGAGC	TAAGGAAGCT
301	AAAATGGAGA	AAAAATCAC	TGGATATACC	ACCGTTGATA	TATCCCAATG	GCATCGTAAA
361	GAACATTTTG	AGGCATTTCA	GTCAGTTGCT	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG
421	GATATTACGG	CCTTTTAA	GACCGTAAAG	AAAAATAAGC	ACAAGTTTTA	TCCGGCCTTT
481	ATTCACATTC	TTGCCCGCCT	GATGAATGCT	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC
541	GGTGAGCTGG	TGATATGGGA	TAGTGTTTAC	CCTTGTTTACA	CCGTTTTCCA	TGAGCAAAC
601	GAAACGTTTT	CATCGCTCTG	GAGTGAATAC	CACGACGATT	TCCGGCAGTT	TCTACACATA
661	TATTCGCAAG	ATGTGGCGTG	TTACGGTGAA	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT
721	GAGAATATGT	TTTTCGTCTC	AGCCAATCCC	TGGGTGAGTT	TCACCAAGTT	TGATTTAAAC
781	GTGGCCAATA	TGGACAACCT	CTTCGCCCCC	GTTTTACCA	TGGGCAAATA	TTATACGCAA
841	GGCGACAAGG	TGCTGATGCC	GCTGGCGATT	CAGGTTTCATC	ATGCCGCTCTG	TGATGGCTTC
901	CATGTCGGCA	GAATGCTTAA	TGAATTACAA	CAGTACTGCG	ATGAGTGGCA	GGGCGGGGCG
961	TAAACGCGTG	GATCCGCTT	ACTAAAAGCC	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT
1021	TTTTGCGGTA	TAAGAATATA	TACTGATATG	TATACCCGAA	GTATGTCAA	AAGAGGTGTG
1081	CTATGAAGCA	GCGTATTACA	GTGACAGTTG	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT
1141	ATATGATGTC	AATATCTCCG	GTCTGGTAAG	CACAACCATG	CAGAATGAAG	CCCGTCTGCT
1201	GCGTGCCGAA	CGCTGGAAAG	CGGAAAATCA	GGAAGGGATG	GCTGAGGTCTG	CCCGGTTTAT
1261	TGAAATGAAC	GGCTCTTTTG	CTGACGAGAA	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT
1321	ACACCTATAA	AAGAGAGAGC	CGTTATCGTC	TGTTTGTGGA	TGTACAGAGT	GATATTATTG
1381	ACACGCCCGG	GCGACGGATG	GTGATCCCCC	TGGCCAGTGC	ACGCTCTGCTG	TCAGATAAAG
1441	TCTCCCGTGA	ACTTTACCCG	GTGGTGATA	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA
1501	CCGATATGGC	CAGTGTGCCG	GTCTCCGTTA	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC
1561	GCGAAAATGA	CATCAAAAAC	GCCATTAAAC	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT
1621	CCCTTATACA	CAGCCAGTCT	GCAGGTTCGAC	CATAGTGAAT	GGATATGTTG	TGTTTTACAG
1681	TATTATGTAG	TCTGTTTTTT	ATGCAAAAATC	TAATTTAATA	TATTGATATT	TATATCATTT
1741	TACGTTTCTC	GTTTCAGCTTT	CTTGATACAA	GTGGTGATTA	TGTCCCTAT	ACTAGGTTAT
1801	TGGAAAATTA	AGGGCCTTGT	GCAACCCACT	CGACTTCTTT	TGGAATATCT	TGAAGAAAAA
1861	TATGAAGAGC	ATTTGTATGA	GCGCGATGAA	GGTGATAAAT	GGCGAAACAA	AAAGTTTGAA
1921	TTGGGTTTGG	AGTTTCCCAA	TCTTCCTTAT	TATATTGATG	GTGATGTTAA	ATTAACACAG
1981	TCTATGGCCA	TCATACGTTA	TATAGCTGAC	AAGCACAACA	TGTTGGGTGG	TTGTCCAAAA
2041	GAGCGTGCG	AGATTTCAAT	GCTTGAAGGA	GCGGTTTTTG	ATATTAGATA	CGGTGTTTCG
2101	AGAATTGCGT	ATAGTAAAGA	CTTGAAACT	CTCAAAGTTG	ATTTTCTTAG	CAAGCTACCT
2161	GAAATGCTGA	AAATGTTTGA	AGATCGTTTA	TGTCATAAAA	CATATTTTAA	TGGTGATCAT
2221	ATAACCCATC	CTGACTTCAT	GTTGTATGAC	GCTCTTGATG	TTGTTTTTATA	CATGACCCCA
2281	GTGTGCCTGG	ATGCGTTCCC	AAAATTAGTT	TGTTTTAAAA	AACGTATTGA	AGCTATCCCA
2341	CAAATTGATA	AGTACTTGAA	ATCCAGCAAG	TATATAGCAT	GGCCTTTGCA	GGGCTGGCAA
2401	GCCACGTTTG	GTGGTGGCGA	CCATCCTCCA	AAATCGGATC	TGGTTCCGCG	TCCATGGGGA
2461	TCCGGCTGCT	AACAAAGCCC	GAAAGGAAGC	TGAGTTGGCT	GCTGCCACCG	CTGAGCAATA
2521	ACTAGCATAA	CCCCTTGGGG	CCTCTAAACG	GGTCTTGAGG	GGTTTTTTGC	TGAAAGGAGG
2581	AACTATATCC	GGATATCCAC	AGGACGGGTG	TGGTCGCCAT	GATCGCGTAG	TCGATAGTGG
2641	CTCCAAGTAG	CGAAGCGAGC	AGGACTGGGC	GGCGGCCAAA	GCGGTCGGAC	AGTGCTCCGA-

FIGURE 44B

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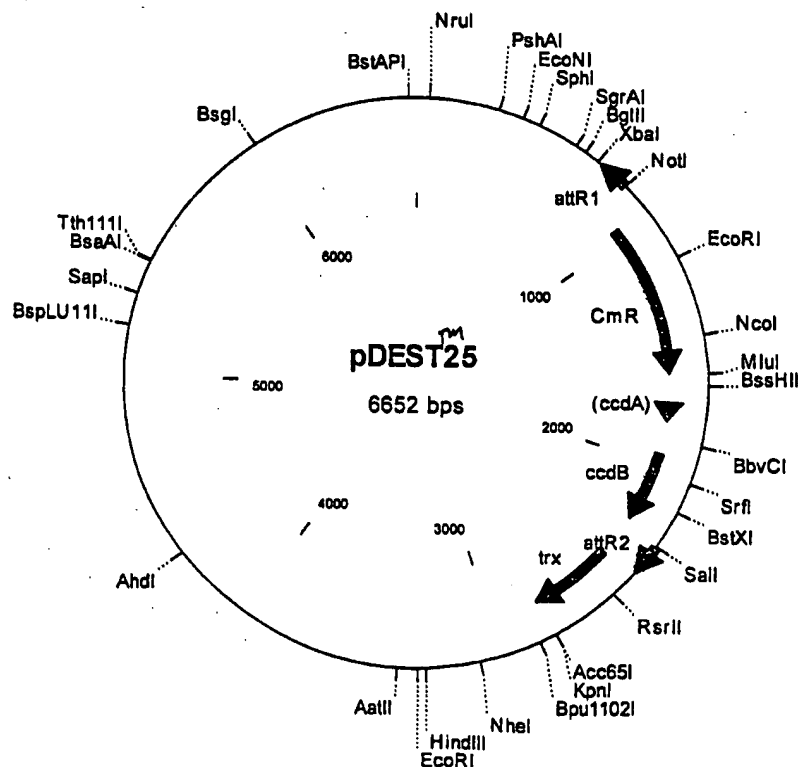
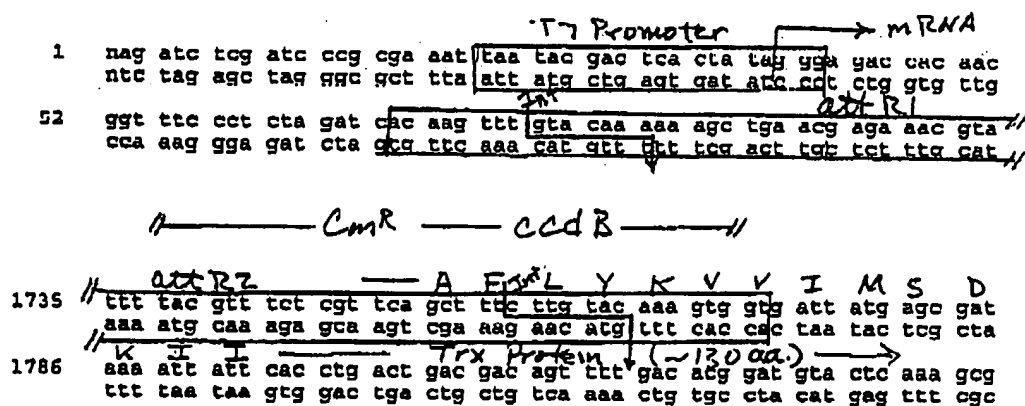
2701 GAACGGGTGC GCATAGAAAT TGCATCAACG CATATAGCGC TAGCAGCACG CCATAGTGAC  
2761 TGGCGATGCT GTCGGAATGG ACGATATCCC GCAAGAGGCC CGGCAGTACC GGCATAACCA  
2821 AGCCTATGCC TACAGCATCC AGGGTGACGG TGGCGAGGAT GACGATGAGC GCATTGTTAG  
2881 ATTTCATACA CGGTGCCTGA CTGCGTTAGC AATTCTTAACTG TGATAAACTA CCGCATTAAA  
2941 GCTTATCGAT GATAAGCTGT CAAACATGAG AATTCTTGAA GACGAAAGGG CCTCGTGATA  
3001 CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT  
3061 TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG  
3121 TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT  
3181 ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT  
3241 GTTTTGTCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA  
3301 CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAAG TCCTTGAGAG TTTTCGCCCC  
3361 GAAGAACGTT TTCCAATGAT GCAACTCTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC  
3421 CGTGTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG  
3481 GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA  
3541 TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC  
3601 GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT  
3661 GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG  
3721 CCTGCAGCAA TGGCAACAAC GTTGCSCAAA CTATTAAGTG GCGAACTACT TACTCTAGCT  
3781 TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC  
3841 TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA CGGTGGGTCT  
3901 CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC  
3961 ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC  
4021 TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT  
4081 TTTAAACTTC ATTTTAAAT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG  
4141 ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC  
4201 AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA  
4261 CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG  
4321 GTAACCTGGT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA  
4381 GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA  
4441 CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGAGACTC AAGACGATAG  
4501 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA GCCCAGCTTG  
4561 GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG  
4621 CTTCGGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG  
4681 CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC  
4741 CTAACCTGGT TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA  
4801 AACGCCAGCA ACGCGGCCCTT TTACCGTTT CTTGGCCTTT GCTGGCCTTT TGCTCACATG  
4861 TTCTTCTCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT  
4921 GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA  
4981 GAGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATATAT  
5041 GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT ACACTCCGCT  
5101 ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC  
5161 CTGACGGGCT TGTCTGCTCC CGGCATCCCG TTACAGACAA GCTGTGACCG TCTCCGGGAG  
5221 CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGGCAGC TGCGGTAAAG  
5281 CTCATCAGCG TGGTCGTGAA GCGATTACCA GATGTCTGCC TGTTTCATCCG CGTCCAGCTC  
5341 GTTGAGTTTC TCCAGAAGCG TTAATGTCTG GCTTCTGATA AAGCGGGCCA TGTTAAGGGC  
5401 GGTTTTTTCC TGTTTGGTCA CTGATGCCTC CGTGTAAAGG GGATTTCTGT TCATGGGGGT  
5461 AATGATACCG ATGAAACGAG AGAGGATGCT CACGATACCG GTTACTGATG ATGAACATGC  
5521 CCGGTTACTG GAACGTTGTG AGGGTAAACA ACTGGCGGTA TGGATGCGGC GGGACCAGAG  
5581 AAAAATCACT CAGGGTCAAT GCCAGCGCTT CGTTAATACA GATGTAGGTG TTCCACAGGG  
5641 TAGCCAGCAG CATCCTGCCA TGCAGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG  
5701 CGTTTCCAGA CTTTACGAAA CACGGAAACC GAAGACCATT CATGTTGTTG CTCAGTCCG  
5761 AGACGTTTTG CAGCAGCAGT CGCTTACAGT TCGCTCGCGT ATCGGTGATT CATTCTGCTA  
5821 ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCTCAAC GACAGGAGCA CGATCATGCG  
5881 CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCGC GTGCGGCTGC TGGAGATGGC  
5941 GGACCGGATG GATATGTTCT GCCAAGGGTT GGTTTGCSCA TTCACAGTTC TCCGCAAGAA  
6001 TTGATTGGCT CCAATTCTTG GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC GGCTTCCATT  
6061 CAGGTCGAGG TGGCCCGGCT CCATGCACCG CGACGCAACG CGGGGAGGCA GACAAGGTAT  
6121 AGGGCGGCGC CTACAATCCA TGCCAACCCG TTCCATGTGC TCGCCGAGGC GGCATAAATC-

FIGURE 44C

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6181 GCCGTGACGA TCAGCGGTCC AGTGATCGAA GTTAGGCTGG TAAGAGCCGC GAGCGATCCT  
6241 TGAAGCTGTC CCTGATGGTC GTCATCTACC TGCCTGGACA GCATGGCCTG CAACGCGGGC  
6301 ATCCCGATGC CGCCGGAAGC GAGAAGAATC ATAATGGGGA AGGCCATCCA GCCTCGCGTC  
6361 GCGAACGCCA GCAAGACGTA GCCCAGCGCG TCGGCCGCCA TGCCGGCGAT AATGGCCTGC  
6421 TTCTCGCCGA AACGTTTGGT GGCGGGACCA GTGACGAAGG CTTGAGCGAG GCGGTGCAAG  
6481 ATTCCGAATA CCGCAAGCGA CAGGCCGATC ATCGTCGCGC TCCAGCGAAA GCGGTCCTCG  
6541 CCGAAAATGA CCCAGAGCGC TGCCGGCACCC TGTCTACGA GTTGATGAT AAAGAAGACA  
6601 GTCATAAGTG CGGCGACGAT AGTCATGCCC CGCGCCCACC GGAAGGAGCT GACTGGGTTG  
6661 AAGGCTCTCA AGGGCATCGG TCGATCGACG CTCTCCCTTA TGCGACTCCT GCATTAGGAA  
6721 GCAGCCCAAGT AGTAGGTTGA GGCCGTTGAG CACCGCCGCC GCAAGGAATG GTGCATGCAA  
6781 GGAGATGGCG CCCAACAGTC CCCCAGCCAC GGGGCCTGCC ACCATACCCA CGCCGAAACA  
6841 AGCGCTCATG AGCCCGAAGT GGCAGAGCCG ATCTTCCCCA TCGGTGATGT CGGCGATATA  
6901 GCGGCCAGCA ACCGCACCTG TGGCGCCGGT GATGCCGGCC ACGATGCGTC CGGCGTAGAG  
6961 G

FIGURE 44B

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FIGURE 45ApDEST25  
Thioredoxin carboxy-fusion vector, T7 promoter

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## pDEST25 6652 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
844..720		attR1
953..1612		CmR
1732..1816		inactivated ccdA
1954..2259		ccdB
2300..2424		attR2
2432..2794		trx

1	CCGGAAGCGA	GAAGAATCAT	AATGGGGAAG	GCCATCCAGC	CTCGCGTCGC	GAACGCCAGC
61	AAGACGTAGC	CCAGCGCGTC	GGCCGCCATG	CCGGCGATAA	TGGCCTGCTT	CTCGCCGAAA
121	CGTTTGGTGG	CGGGACCAGT	GACGAAGGCT	TGAGCGAGGG	CGTGCAAGAT	TCCGAATACC
181	GCAAGCGACA	GGCCGATCAT	CGTCGCGCTC	CAGCGAAAGC	GGTCCTCGCC	GAAAAAGACC
241	CAGAGCGCTG	CCGGCACCTG	TCCTACGAGT	TGCATGATAA	AGAAGACAGT	CATAAGTGCG
301	GCGACGATAG	TCATGCCCCG	CGCCACCCGG	AAGGAGCTGA	CTGGGTTGAA	GGCTCTCAAG
361	GGCATCGGTC	GATCGACGCT	CTCCCTTATG	CGACTCCTGC	ATTAGGAAGC	AGCCCAGTAG
421	TAGGTTGAGG	CCGTTGAGCA	CCGCCGCCGC	AAGGAATGGT	GCATGCAAGG	AGATGGCGCC
481	CAACAGTCCC	CCGGCCACGG	GGCTGCCAC	CATACCCACG	CCGAAACAAG	CGCTCATGAG
541	CCCGAAGTGG	CGAGCCCGAT	CTTCCCCATC	GGTGATGTCT	GCGATATAGG	CGCCAGCAAC
601	CGCACCTGTG	GCGCCGGTGA	TGCCGCGCAC	GATGCGTCCG	GCGTAGAGGA	TGCAGATCTC
661	GATCCCGCGA	AATTAATACG	ACTCACTATA	GGGAGACCAC	AACGGTTTCC	CTCTAGATCA
721	CAAGTTTGTA	CAAAAAAGCT	GAACGAGAAA	CGTAAAATGA	TATAAATATC	AATATATTAA
781	ATTAGATTTT	GCATAAAAAA	CAGACTACAT	AATACTGTAA	AACACAACAT	ATCCAGTCAC
841	TATGGCGGCC	GCATTAGGCA	CCCCAGGCTT	TACACTTTAT	GCTTCCGGCT	CGTATAATGT
901	GTGGATTTTG	AGTTAGGATC	CGGCGAGATT	TTCAGGAGCT	AAGGAAGCTA	AAATGGAGAA
961	AAAAATCACT	GGATATACCA	CCGTTGATAT	ATCCCAATGG	CATCGTAAAG	AACATTTTGA
1021	GGCATTTCAG	TCAGTTGCTC	AATGTACCTA	TAACCAGACC	GTTTCAGCTGG	ATATTACGGC
1081	CTTTTAAAG	ACCGTAAAGA	AAAATAAGCA	CAAGTTTAT	CCGGCCCTTA	TTCACATTCT
1141	TGCCCCGCTG	ATGAATGCTC	ATCCGGAATT	CCGTATGGCA	ATGAAAGACG	GTGAGCTGGT
1201	GATATGGGAT	AGTGTTACAC	CTTGTTACAC	CGTTTCCAT	GAGCAAACCTG	AAACGTTTTTC
1261	ATCGCTCTGG	AGTGAATACC	ACGACGATTT	CCGGCAGTTT	CTACACATAT	ATTTCGAAGA
1321	TGTGGCGTGT	TACGGTGAAA	ACCTGGCCTA	TTTCCCTAAA	GGGTTTATTG	AGAATATGTT
1381	TTTCGTCTCA	GCCAATCCCT	GGGTGAGTTT	CACCAAGTTT	GATTAAACG	TGGCCAATAT
1441	GGACAACTTC	TTCCGCCCCG	TTTTACCAT	GGGCAAATAT	TATACGCAAG	GCGACAAGGT
1501	GCTGATGCCG	CTGGCGATTG	AGGTTTCATCA	TGCCGCTCTG	GATGGCTTCC	ATGTCGGCAG
1561	AATGCTTAAT	GAATTACAAC	AGTACTGCCA	TGAGTGGCAG	GGCGGGGCGT	AAACGCGTGG
1621	ATCCGGCTTA	CTAAAAGCCA	GATAACAGTA	TGCGTATTTG	CGCGCTGATT	TTTGGCGGTAT
1681	AAGAAATATAT	ACTGATATGT	ATACCCGAAG	TATGTCAAAA	AGAGGTGTGC	TATGAAGCAG
1741	CGTATTACAG	TGACAGTTGA	CAGCGACAGC	TATCAGTTGC	TCAAGGCATA	TATGATGTCA
1801	ATATCTCCGG	TCTGGTAAGC	ACAACCATGC	AGAATGAAGC	CCGTCGCTCG	CGTGCCGAAC
1861	GCTGGAAAGC	GGAAAATCAG	GAAGGGATGG	CTGAGGTCCG	CCGGTTTATT	GAAATGAACG
1921	GCTCTTTTGC	TGACGAGAAC	AGGGACTGGT	GAAATGCAGT	TTAAGGTTTA	CACCTATAAA
1981	AGAGAGAGCC	GTTATCGTCT	GTTTGTGGAT	GTACAGAGTG	ATATTATTGA	CACGCCCCGG
2041	CGACGGATGG	TGATCCCCCT	GGCCAGTGCA	CGTCTGCTGT	CAGATAAAGT	CTCCCGTGAA
2101	CTTTACCCGG	TGGTGATAT	CGGGGATGAA	AGCTGGCGCA	TGATGACCAC	CGATATGGCC
2161	AGTGTGCCGG	TCTCCGTTAT	CGGGGAAGAA	GTGGCTGATC	TCAGCCACCG	CGAAAATGAC
2221	ATCAAAAACG	CCATTAACTT	GATGTTCTGG	GGAATATAAA	TGTCAGGCTC	CCTTATACAC
2281	AGCCAGTCTG	CAGGTCGACC	ATAGTGACTG	GATATGTTGT	GTTTTACAGT	ATTATGTAGT
2341	CTGTTTTTTA	TGCAAAATCT	AATTTAATAT	ATTGATATTT	ATATCATTTT	ACGTTTCTCG
2401	TTTCAGCTTC	TTGTACAAAG	TGGTGATTAT	GAGCGATAAA	ATTATTACAC	TGACTGACGA
2461	CAGTTTTTGAC	ACGGATGTAC	TCAAAGCGGA	CGGGGCGATC	CTCGTCGATT	TCTGGGCAGA
2521	GTGGTGCGGT	CCGTGCAAAA	TGATCGCCCC	GATTCTGGAT	GAAATCGCTG	ACGAATATCA
2581	GGGCAAACTG	ACCGTTGCAA	AACTGAACAT	CGATCAAAAC	CCTGGCACTG	CGCCGAAATA
2641	TGGCATCCGT	GGTATCCCGA	CTCTGCTGCT	GTTCAAAAAC	GGTGAAGTGG	CGGCAACCAA
2701	AGTGGGTGCA	CTGTCTAAAG	GTCAGTTGAA	AGAGTTCTCT	GACGCTAACC	TGGCCGTTTC
2761	TGGTTCTGGT	GATGACGATG	ACAAGGTACC	CGGGGATCGA	TCCGGCTGCT	AACAAAGCCC

FIGURE 45B

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2821 GAAAGGAAGC TGAGTTGGCT GCTGCCACCG CTGAGCAATA ACTAGCATAA CCCCTTGGGG  
2881 CCTCTAAACG GGTCTTGAGG GGTTTTTTGC TGAAAGGAGG AACTATATCC GGATATCCAC  
2941 AGGACGGGTG TGGTCGCCAT GATCGCGTAG TCGATAGTGG CTCCAAGTAG CGAAGCGAGC  
3001 AGGACTGGGC GGCGGCCAAA GCGGTCGGAC AGTGCTCCGA GAACGGGTGC GCATAGAAAT  
3061 TGCATCAACG CATATAGCGC TAGCAGCACG CCATAGTGAC TGGCGATGCT GTCGGAATGG  
3121 ACGATATCCC GCAAGAGGCC CGGCAGTACC GGCATAACCA AGCCTATGCC TACAGCATCC  
3181 AGGGTGACGG TGCCGAGGAT GACGATGAGC GCATTGTTAG ATTTTCATACA CGGTGCCTGA  
3241 CTGCGTTAGC AATTTAACTG TGATAAACTA CCGCATTAAA GCTTATCGAT GATAAGCTGT  
3301 CAAACATGAG AATTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA  
3361 TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCCGGGAA ATGTGCGCGG  
3421 AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA  
3481 ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG  
3541 TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTGTCTC ACCCAGAAAC  
3601 GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAAGT  
3661 GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT  
3721 GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTGTGACG CCGGCAAGA  
3781 GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC  
3841 AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT  
3901 GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC  
3961 CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT  
4021 GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA TGGCAACAAC  
4081 GTTGCGCAAA CTATTAACCTG GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA  
4141 CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CCGCTGGCTG  
4201 GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT  
4261 GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC  
4321 TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA  
4381 ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAACCTTC ATTTTAAATT  
4441 TAAAGGATC TAGGTGAAGA TCCTTTTGA TAATCTCATG ACCAAAATCC CTTAAGCTGA  
4501 GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC  
4561 TTTTCTCTG CGCGTAATCT GAGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGTGGT  
4621 TTGTTTGGCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC  
4681 GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC  
4741 TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG  
4801 CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG  
4861 GTCGGGCTGA ACGGGGGGTT CGTGACACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA  
4921 ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC  
4981 GGACAGGTAT CCGGTAAGCG GCAGGTCGG AACAGGAGAG CGCAGGAGG AGCTTCCAGG  
5041 GGGAAACGCC TGGTATCTTT ATAGTCTGTG CGGGTTTCGC CACCTCTGAC TTGAGCGTCG  
5101 ATTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGAAA AACGCCAGCA ACGCGGCCCT  
5161 TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTCTCTG CGTTATCCCC  
5221 TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG  
5281 AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCTGA TGCGGTATTT  
5341 TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATATAT GGTGCACTCT CAGTACAATC  
5401 TGCTCTGATG CCGCATAGTT AAGCCAGTAT AACTCCGCT ATCGCTACGT GACTGGGTCA  
5461 TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC  
5521 CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT  
5581 CACCGTCATC ACCGAAACGC GCGAGGCAGC TGCGGTAAAG CTCATCAGCG TGGTCGTGAA  
5641 GCGATTACAA GATGTCTGCC TGTTTCATCCG CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG  
5701 TTAATGTCTG GCTTCTGATA AAGCGGGCCA TGTTAAGGGC GGTTTTTTCC TGTGTTGTC  
5761 CTGATGCCCTC CGTGTAAGGG GGATTTCTGT TCATGGGGGT AATGATACCG ATGAAACGAG  
5821 AGAGGATGCT CACGATACGG GTTACTGATG ATGAACATGC CCGGTTACTG GAACGTTGTG  
5881 AGGGTAAACA ACTGGCGGTA TGGATGCGGC GGGACCAGAG AAAAATCACT CAGGGTCAAT  
5941 CGCAGCGCTT CGTTAATACA GATGTAGGTG TTCCACAGGG TAGCCAGCAG CATCCTGCGA  
6001 TGCAGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG CGTTTCCAGA CTTTACGAAA  
6061 CACGGAAACC GAAGACCATT CATGTTGTTG CTCAGGTCGC AGACGTTTTG CAGCAGCAGT  
6121 CGCTTACAGT TCGCTCGCGT ATCGGTGATT CATTCTGCTA ACCAGTAAGG CAACCCCGCC  
6181 AGCCTAGCCG GGTCTCAAC GACAGGAGCA CGATCATGCG CACCCGTGGC CAGGACCCAA  
6241 CGCTGCCCGA GATGCGCCGC GTGCGGCTGC TGGAGATGGC GGACGCGATG GATATGTTCT-

FIGURE 45C

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6301 GCCAAGGGTT GGTTTGC GCA TTCACAGTTC TCCGCAAGAA TTGATTGGCT CCAATTCTTG  
6361 GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC GGCTTCCATT CAGGTCGAGG TGGCCCGGCT  
6421 CCATGCACCG CGACGCAACG CGGGGAGGCA GACAAGGTAT AGGGCGGGCG CTACAATCCA  
6481 TGCCAACCCG TTCCATGTGC TCGCCGAGGC GGCATAAATC GCCGTGACGA TCAGCGGTCC  
6541 AGTGATCGAA GTTAGGCTGG TAAGAGCCGC GAGCGATCCT TGAAGCTGTC CCTGATGGTC  
6601 GTCATCTACC TGCCTGGACA GCATGGCCTG CAACGCGGGC ATCCCGATGC CG

FIGURE 45D

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FIGURE 46A

# pDEST26 His6 Amino Fusion in pCMV Sport-neo Vector

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600   ttg acg tca atg gga gtt tgt ttt ggc acc aaa atc aac ggg act ttc caa
      aac tgc agt tac cct caa aca aaa ccg tgg ttt tag ttg ccc tga aag gtc

651   aat gtc gta aca act ccg ccc oat tga cgc aaa tgg gcg gta ggc gtg tac
      tta cag cat tgt tga ggc ggg gta act ggc ttt acc cgc cat ccg cac atg

702   // ggt ggg agg tct ata taa gaa gag ctc gtt tag tga acc gtc aga tgg cct
      //cca ccc tcc aga tat att cgt ctc gag caa atc act tgg cag tct ago gga

753   gga gac ggc atc cac gct gtt ttg acc tcc ata gaa gac acc ggg acc gat
      cct ctg cgg tag gtg cga caa aac tgg agg tat ott ctg tgg ccc tgg cta

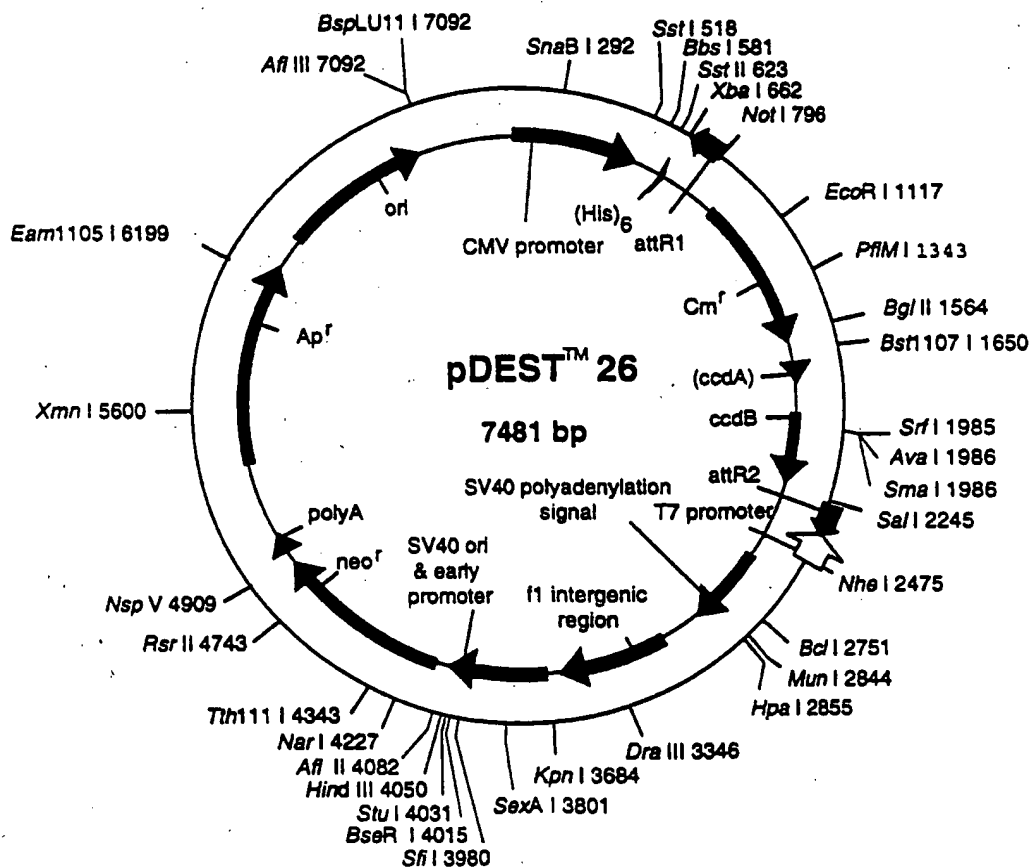
804   cca gcc tcc gga ctc tag cct agg ccg cgg acc atg gcg tac tac cat cac
      ggt cgg agg cct gag atc gga tcc ggc gcc tgg tac cgc atg atg gta gtc

855   H H H H S R S T S V K K A
      cat cac cat cac tct aga tca aca agt ttg tac aaa aaa gct gaa cga gaa
      gta gtg gta gtg aga tct agt tgt tca aac atg ttt ttt cga ott gct ctt
  
```

CMV Promoter → MRNA

Start Transl →

Int V





## pDEST26 7481 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
492..509		his6
619..519		attR1
752..1411		CmR
1531..1615		inactivated ccdA
1753..2058		ccdB
2099..2223		attR2
2409..2771		SV40 polyA
2966..3421		f1 intergenic region
3485..3903		SV40 promoter
3948..4742		neo
4806..4854		polyA
5265..6125		Apr
6274..6913		ori
7344..385		CMV promoter

1	GTAAACTGCC	CACTTGGCAG	TACATCAAGT	GTATCATATG	CCAAGTACGC	CCCCTATTGA
61	CGTCAATGAC	GGTAAATGGC	CCGCCTGGCA	TTATGCCCAG	TACATGACCT	TATGGGACTT
121	TCCTACTTGG	CAGTACATCT	ACGTATTAGT	CATCGCTATT	ACCATGGTGA	TGCGGTTTGT
181	GCAGTACATC	AATGGGCGTG	GATAGCGGTT	TGACTCACGG	GGATTTCCAA	GTCTCCACCC
241	CATTGACGTC	AATGGGAGTT	TGTTTTGGCA	CCAAAATCAA	CGGGACTTTC	CAAAATGTCG
301	TAACAACTCC	GCCCCATTGA	CGCAAATGGG	CGGTAGGCGT	GTACGGTGGG	AGGTCTATAT
361	AAGCAGAGCT	CGTTTAGTGA	ACCGTCAGAT	CGCCTGGAGA	CGCCATCCAC	GCTGTTTTGA
421	CCTCCATAGA	AGACACCGGG	ACCGATCCAG	CCTCCGGACT	CTAGCCTAGG	CCGCGGACCA
481	TGGCGTACTA	CCATCACCAT	CACCATCACT	CTAGATCAAC	AAGTTTGTAC	AAAAAAGCTG
541	AACGAGAAAC	GTAAAATGAT	ATAAATATCA	ATATATTAAA	TTAGATTTTG	CATAAAAAAC
601	AGACTACATA	ATACTGTAAA	ACACAACATA	TCCAGTCACT	ATGGCGGCCG	CATTAGGCAC
661	CCCAGGCTTT	ACACTTTATG	CTTCCGGCTC	GTATAATGTG	TGGATTTTGA	GTTAGGATCC
721	GGCGAGATTT	TCAGGAGCTA	AGGAAGCTAA	AATGGAGAAA	AAAATCACTG	GATATACCAC
781	CGTTGATATA	TCCCAATGGC	ATCGTAAAGA	ACATTTTGAG	GCATTTCACT	CAGTTGCTCA
841	ATGTACCTAT	AACCAGACCG	TTCAGCTGGA	TATTACGGCC	TTTTTAAAGA	CCGTAAAGAA
901	AAATAAGCAC	AAGTTTTATC	CGGCCTTTAT	TCACATTCTT	GCCCGCCTGA	TGAATGCTCA
961	TCCGGAATTC	CGTATGGCAA	TGAAAGACGG	TGAGCTGGTG	ATATGGGATA	GTGTTCAACC
1021	TTGTTACACC	GTTTTCCATG	AGCAAACCTGA	AACGTTTTCA	TCGCTCTGGA	GTGAATACCA
1081	CGACGATTTT	CGGCAGTTTC	TACACATATA	TTCCGCAAGAT	GTGGCGTGTT	ACGGTGAAAA
1141	CCTGGCCTAT	TTCCCTAAAG	GGTTTATTGA	GAATATGTTT	TTCTCTCAG	CCAATCCCTG
1201	GGTGAGTTTC	ACCAGTTTTG	ATTTAAACGT	GGCCAATATG	GACAACTTCT	TCGCCCCCGT
1261	TTTCACCATG	GGCAAATATT	ATACGCAAGG	CGACAAGGTG	CTGATGCCCG	TGGCGATTCA
1321	GGTTCATCAT	GCCGTCTGTG	ATGGCTTCCA	TGTCGGCAGA	ATGCTTAATG	AATTACAACA
1381	GTA CTGCGAT	GAGTGGCAGG	GCGGGGCGTA	AAGATCTGGA	TCCGGCTTAC	TAAAAGCCAG
1441	ATAACAGTAT	GCGTATTTGC	GCGCTGATTT	TTGCGGTATA	AGAATATATA	CTGATATGTA
1501	TACCGAAGT	ATGTCAAAAA	GAGGTGTGCT	ATGAAGCAGC	GTATTACAGT	GACAGTTGAC
1561	AGCGACAGCT	ATCAGTTGCT	CAAGGCATAT	ATGATGTCAA	TATCTCCGGT	CTGGTAAGCA
1621	CAACCATGCA	GAATGAAGCC	CGTCGTCTGC	GTGCCGAACG	CTGGAAGCG	GAAAATCAGG
1681	AAGGGATGGC	TGAGGTCGCC	CGGTTTATTG	AAATGAACGG	CTCTTTTGCT	GACGAGAACA
1741	GGGACTGGTG	AAATGCAGTT	TAAGGTTTAC	ACCTATAAAA	GAGAGAGCCG	TTATCGTCTG
1801	TTTGTGGATG	TACAGAGTGA	TATTATTGAC	ACGCCCCGGC	GACGGATGGT	GATCCCCCTG
1861	GCCAGTGAC	GTCTGCTGTC	AGATAAAGTC	TCCCGTGAAC	TTTACCCGGT	GGTGATATC
1921	GGGGATGAAA	GCTGGCGCAT	GATGACCACC	GATATGGCCA	GTGTGCCGGT	CTCCGTTATC
1981	GGGGAAGAAG	TGGCTGATCT	CAGCCACCGC	GAAAATGACA	TCAAAAACGC	CATTAACTTG
2041	ATGTTCTGGG	GAATATAAAT	GTCAGGCTCC	CTTATACACA	GCCAGTCTGC	AGGTCGACCA
2101	TAGTGACTGG	ATATGTTGTG	TTTTACAGTA	TTATGTAGTC	TGTTTTTTAT	GCAAAATCTA
2161	ATTTAATATA	TTGATATTTA	TATCATTTTA	CGTTTCTCGT	TCAGCTTTCT	TGTACAAAGT
2221	GGTTGATCGC	GTGCATGCGA	CGTCATAGCT	CTCTCCCTAT	AGTGAGTCGT	ATTATAAGCT
2281	AGGCACTGGC	CGTCGTTTTA	CAACGTCGTG	ACTGGGAAAA	CTGCTAGCTT	GGGATCTTTG -

FIGURE 46B

2341 TGAAGGAACC TTACTTCTGT GGTGTGACAT AATTGGACAA ACTACCTACA GAGATTTAAA  
2401 GCTCTAAGGT AAATATAAAA TTTTAAAGTG TATAATGTGT TAAACTAGCT GCATATGCTT  
2461 GCTGCTTGAG AGTTTGTGCTT ACTGAGTATG ATTTATGAAA ATATTATACA CAGGAGCTAG  
2521 TGATTCTAAT TGTTTGTGTA TTTTAGATTG ACAGTCCCAA GGCTCATTTT AGGCCCTCA  
2581 GTCCTCACAG TCTGTTTCATG ATCATAATCA GCCATACCAC ATTTGTAGAG GTTTTACTTG  
2641 CTTTAAAAAA CCTCCACAC CTCCCCCTGA ACCTGAAACA TAAATGAAT GCAATTGTTG  
2701 TTGTTAACTT GTTTATTGCA GCTTATAATG GTTACAAATA AAGCAATAGC ATCACAAAT  
2761 TCACAAATAA AGCATTTTTT TCACTGCATT CTAGTTGTGG TTGTGCCAAA CTCATCAATG  
2821 TATCTTATCA TGTCTGGATC GATCCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC  
2881 GGTTTGGCGTA TTGGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC TTCCCAACAG  
2941 TTGCCGAGCC TGAATGGCGA ATGGGACGCG CCCTGTAGCG GCGCATTAAG CGCGGCGGGT  
3001 GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCTAGCGCC CGCTCCTTTC  
3061 GCTTCTTCTT CCTCCTTCTT CGCCACGTTT GCCGGCTTTC CCCGTCAAGC TCTAAATCGG  
3121 GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT  
3181 TAGGGTGTAG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG  
3241 TTGGAGTCCA CGTCTTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC ACTCAACCCT  
3301 ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCTA TTTCCGCCTA TTGGTTAAAA  
3361 AATGAGCTGA TTTAACAAAT ATTTAACGCG AATTTTAAACA AAATATTAAC GTTTACAATT  
3421 TCGCCTGATG CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACC GCATACGCGG  
3481 ATCTGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACCTTGTT AGGTACCTTC  
3541 TGAGGCGGAA AGAACCAGCT GTGGAATGTG TGTCAAGTAG GGTGTGGAAA GTCCCCAGGC  
3601 TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA  
3661 AAGTCCCCAG GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA  
3721 ACCATAGTCC CGCCCCTAAC TCCGCCATC CCGCCCCCTA CTCCGCCAG TTCCGCCCAT  
3781 TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC  
3841 TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC TTTTGTGGAG GCCTAGGCTT TTGCAAAAAG  
3901 CTTGATTCTT CTGACACAAC AGTCTCGAAT TTAAGGCTAG AGCCACCATG ATTGAACAAG  
3961 ATGGATTGCA CGCAGGTTCT CCGGCCGCTT GGGTGGAGAG GCTATTCGGC TATGACTGGG  
4021 CACAACAGAC AATCGGCTGC TCTGATGCCG CCGTGTTCGG GCTGTGAGCG CAGGGGCGCC  
4081 CGGTTCTTTT TGTCAAGACC GACCTGTCCG GTGCCCTGAA TGAAGTCAG GACGAGGCAG  
4141 CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCTTGCGC AGCTGTGCTC GACGTTGTCA  
4201 CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCAGGAT CTCCTGTCTC  
4261 CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA TGCAATGCGG CGGCTGCATA  
4321 CGCTTGATCC GGCTACCTGC CCATTGACG ACCAAGCGAA ACATCGCATC GAGCGAGCAC  
4381 GTACTCGGAT GGAAGCCGGT CTTGTGATCT AGGATGATCT GGACGAAGAG CATCAGGGGC  
4441 TCGCGCCAGC CGAACTGTTT GCCAGGCTCA AGGCGCGCAT GCGGACGGC GAGGATCTCG  
4501 TCGTGACCCA TGGCGATGCC TGCTTGCCGA ATATCATGGT GGAAATGGC CGCTTTTCTG  
4561 GATTTCATCGA CTGTGGCCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA GCGTTGGCTA  
4621 CCCGTGATAT TGCTGAAGAG CTTGGCGGCG AATGGGCTGA CCGCTTCTCT GTGCTTTACG  
4681 GTATCGCCGC TCCCGATTG CAGCGCATCG CCTTCTATCG CCTTCTTGAC GAGTTCTTCT  
4741 GAGCGGGACT CTGGGGTTCG AAATGACCGA CCAAGCGACG CCCAACCTGC CATCACGATG  
4801 GCCGCAATAA AATATCTTTA TTTTCATTAC ATCTGTGTGT TGTTTTTTTG TGTGAATCGA  
4861 TAGCGATAAG GATCCGCGTA TGGTGCACTC TCAGTACAAT CTGCTCTGAT GCCGCATAGT  
4921 TAAGCCAGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC  
4981 CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT  
5041 CACCGTCATC ACCGAAACGC GCGAGACGAA AGGGCCTCGT GATACGCCTA TTTTATAGG  
5101 TTAATGTCTAT GATAATAATG GTTTCTTAGA CGTCAGGTGG CACTTTTTCG GGAATGTGC  
5161 GCGGAACCCC TATTTGTTTA TTTTCTAAA TACATTCAA TATGTATCCG CTCATGAGAC  
5221 AATAACCTTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCAACATT  
5281 TCCGTGTGCG CCTTATTCCC TTTTGTGCGG CATTTTGCC TCTGTTTTT GCTACCCAG  
5341 AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTACATCG  
5401 AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCCAGAGAA CGTTTTCCAA  
5461 TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCGTATT GACGCCGGG  
5521 AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA CTTGGTTGAG TACTACCAG  
5581 TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA  
5641 CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC  
5701 TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACCTG CCTGATCGT TGGGAACCGG  
5761 AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA -

5821 CAACGTTGCG CAAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA  
5881 TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG  
5941 GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG  
6001 CACTGGGGCC AGATGGTAAG CCTCCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG  
6061 CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT  
6121 GGTAACGTGC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTAAAA CTTCATTTTT  
6181 AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC  
6241 GTGAGTTTTC GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG  
6301 ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCAACG CTACCAGCGG  
6361 TGGTTTGTTT GCCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAAC TGGCTTCAGCA  
6421 GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA  
6481 ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA  
6541 GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC  
6601 AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA  
6661 CCGAACTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA  
6721 AGGCGGACAG GTATCCGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC  
6781 CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGACTTGAGC  
6841 GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG  
6901 CCTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTGTCTCA CATGTTCTTT CCTGCGTTAT  
6961 CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC GCTCGCCGCA  
7021 GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC CCAATACGCA  
7081 AACC GCCTCT CCCC GCCTCT TGGCCGATTC ATTAATGCAG AGCTTGCAAT TCGCGCGTTT  
7141 TTCAATATTA TTGAAGCATT TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTGAA  
7201 GTATTTAGAA AAATAAACAA ATAGGGGTTT CCGCGACATT TCCCCGAAAA GTGCCACCTG  
7261 ACGTCTAAGA AACCATTATT ATCATGACAT TAACCTATAA AAATAGGCGT AGTACGAGGC  
7321 CCTTTCATC ATTAGATGCA TGTGCTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA  
7381 CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA  
7441 ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC G

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FIGURE 47A

# pDEST 27 GST Amino Fusion in pCMV Sport-neo Vector

CMV Promoter

600 // nac ggt ggg agg tct ata taa gca gag ctc gtc tag tga acc gtc aga tcy  
// ntg cca ccc tcc aga tat att cgt ctc gag caa atc act tgg dag tct ago

651 cct gga gac gcc atc cac gct gtt ttg acc tcc ata gaa gac acc ggg acc  
gga cct ctg cgg tag gtg cga caa aac tgg agg tat ctt ctg tgg ccc tgg

702 gat cca gcc tcc gga ctc tag cct agg ccg cgg acc atg gcc cct ata ata  
cta ggt cgg agg cct gag atc gga tcc ggc gcc tgg cag cgg gga tat gat

753 ggt tat tgg aaa att aag ggc ctt gtg caa ccc act oga ott ctt ttg gaa  
cca ata acc ttt taa ttc ccg gaa cac gtt ggg tga gct gaa gaa aac ctt

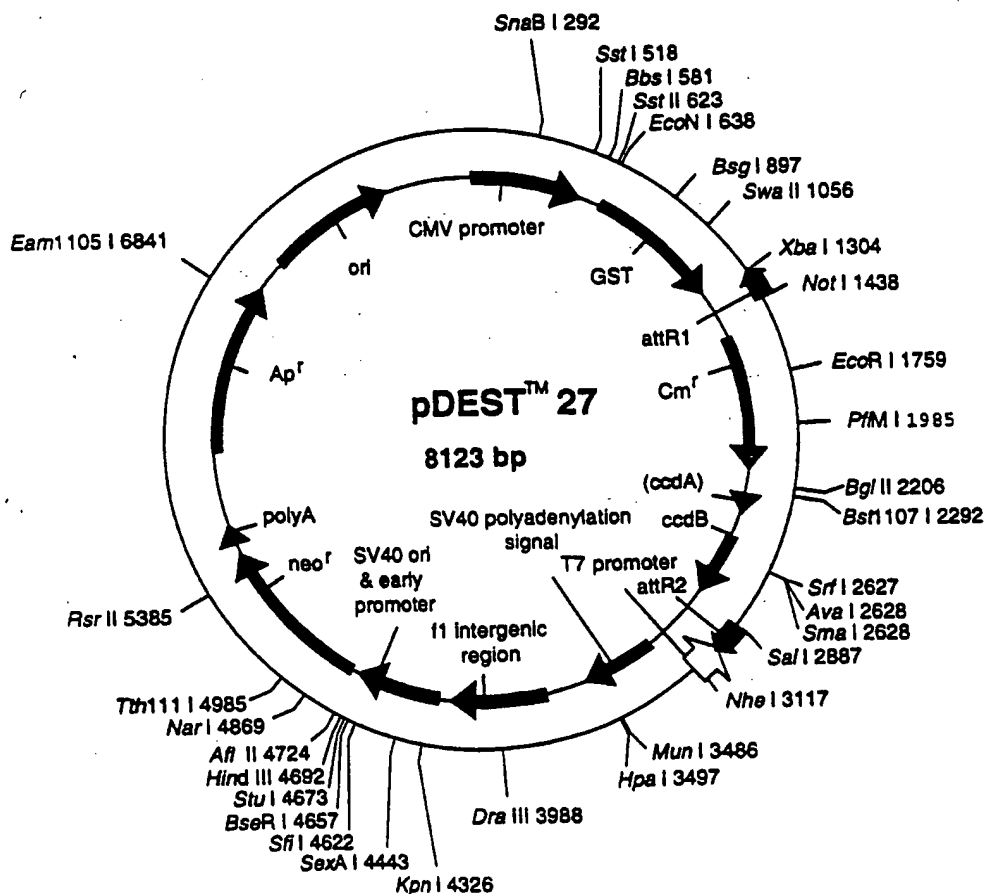
804 tat ctt gaa gaa aaa tat gaa gag cat ttg tat gag cgc gac gaa ggt gat  
ata gaa ctt ctt ttt ata ctt ctc gta aac ata ctc gcg cta ctt cca cta

1365 // ttt ggt ggt ggc gac cat cct cca aaa tgc gat ctg gtt ccg cgt tct aga  
// aaa cca cca ccg ctg gta gga ggt ttt agc cta gac oaa ggc gca aga tct

1416 tca aca agt ttg tac aaa aaa gct gaa cga gaa acg  
agt tgt tca aac atg ttg ttt cga ctt gct att tgc

Start Transl GST

Int attR1



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## pDEST27 8123 bp (rotated to position 7800)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
130..793	GST
803..927	attR1
1036..1695	CmR
1815..1899	inactivated ccdA
2037..2342	ccdB
2383..2507	attR2
2693..3055	SV40 polyA
3250..3705	f1 intergenic region
3769..4187	SV40 promoter
4232..5026	neo
5090..5138	polyA
5549..6409	Apr
6558..7197	ori
7628..27	CMV promoter

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1 ATAAGCAGAG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA GACGCCATCC ACGCTGTTTT
61 GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGGA CTCTAGCCTA GGCCGCGGAC
121 CATGGCCCCCT ATACTAGGTT ATTGGAAGAA TAAGGGCCTT GTGCAACCCA CTCGACTTCT
181 TTTGGAATAT CTTGAAGAAA AATATGAAGA GCATTTGTAT GAGCGCGATG AAGGTGATAA
241 ATGGCGAAAC AAAAAGTTTG AATTGGGTTT GGAGTTTCCC AATCTTCCTT ATTATATTGA
301 TGGTGATGTT AAATTAACAC AGTCTATGGC CATCATACGT TATATAGCTG ACAAGCACAA
361 CATGTTGGGT GGTGTGTCCT AAGAGCGTGC AGAGATTTC AATGCTTGAAG GAGCGGTTTT
421 GGATATTAGA TACGGTGTTC CGAGAATTGC ATATAGTAAA GACTTTGAAA CTCTCAAAGT
481 TGATTTTCTT AGCAAGCTAC CTGAAATGCT GAAATGTTC GAAGATCGTT TATGTCATAA
541 AACATATTTA AATGGTGATC ATGTAACCCA TCCTGACTTC ATGTTGTATG ACGCTCTTGA
601 TGTGTTTTTA TACATGGACC CAATGTGCCT GGATGCGTTC CCAAAATTAG TTTGTTTTAA
661 AAAACGTATT GAAGCTATCC CACAAATTGA TAAGTACTTG AAATCCAGCA AGTATATAGC
721 ATGGCCTTTG CAGGGCTGGC AAGCCACGTT TGGTGGTGGC GACCATCCTC CAAAATCGGA
781 TCTGGTCCG CGTTCTAGAT CAACAAGTTT GTACAAAAAA GCTGAACGAG AAACGTAAAA
841 TGATATAAAT ATCAATATAT TAAATTAGAT TTTGCATAAA AAACAGACTA CATAATACTG
901 TAAACACAA CATATCCAGT CACTATGGCG GCCGCATTAG GCACCCAGG CTTTACACTT
961 TATGCTTCCG GCTCGTATAA TGTGTGGATT TTGAGTTAGG ATCCGCGCAG ATTTTCAGGA
1021 GCTAAGGAAG CTAAATGGA GAAAAAATC ACTGGATATA CCACCGTTGA TATATCCCAA
1081 TGGCATCGTA AAGAACATTT TGAGGCATTT CAGTCAGTTG CTCAATGTAC CTATAACCAG
1141 ACCGTTTCAGC TGGATATTAC GGCCTTTTTTA AAGACCGTAA AGAAAAATAA GCACAAGTTT
1201 TATCCGCGCT TTATTCACAT TCTTGCCCGC CTGATGAATG CTCATCCGGA ATTCCGTATG
1261 GCAATGAAAG ACGGTGAGCT GGTGATATGG GATAGTGTTC ACCCTTGTTA CACCGTTTTT
1321 CATGAGCAAA CTGAAACGTT TTCATCGCTC TGGAGTGAAT ACCACGACGA TTTCCGGCAG
1381 TTTCTACACA TATATTCGCA AGATGTGGCG TGTTACGGTG AAAACCTGGC CTATTTCCCT
1441 AAAGGGTTTA TTGAGAATAT GTTTTTCGTC TCAGCCAATC CCTGGGTGAG TTTCAACAGT
1501 TTTGATTTAA ACGTGGCCAA TATGGACAAC TTCTTCGCCC CCGTTTTTAC CATGGGCAAA
1561 TATTATACGC AAGGCGACAA GGTGCTGATG CCGCTGGCGA TTCAGGTTCA TCATGCCGTC
1621 TGTGATGGCT TCCATGTCGG CAGAATGCTT AATGAATTAC AACAGTACTG CGATGAGTGG
1681 CAGGGCGGGG CGTAAAGATC TGGATCCGGC TTAATAAAG CCAGATAACA GTATGCGTAT
1741 TTGCGCGCTG ATTTTTCGGG TATAAGAATA TATACTGATA TGTATACCCG AAGTATGTCA
1801 AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT TGACAGCGAC AGCTATCAGT
1861 TGCTCAAGGC ATATATGATG TCAATATCTC CGGTCTGGTA AGCACAACCA TGCAGAATGA
1921 AGCCCGCTCGT CTGCGTGCCG AACGCTGGAA AGCGGAAAAT CAGGAAGGGA TGGCTGAGGT
1981 CGCCCGGTTT ATTGAAATGA ACGGCTCTTT TGCTGACGAG AACAGGGACT GGTGAAATGC
2041 AGTTTAAAGT TTACACCTAT AAAAGAGAGA GCCGTTATCG TCTGTTTGTG GATGTACAGA
2101 GTGATATTAT TGACACGCCG GGGCGACGGA TGGTGATCCC CCTGGCCAGT GCACGTCTGC
2161 TGTGATATAA AGTCTCCCGT GAACTTTACC CGGTGGTGCA TATCGGGGAT GAAAGCTGGC
2221 GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA GAAGTGGCTG
2281 ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTT TGGGGAATAT-

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Figure 47B

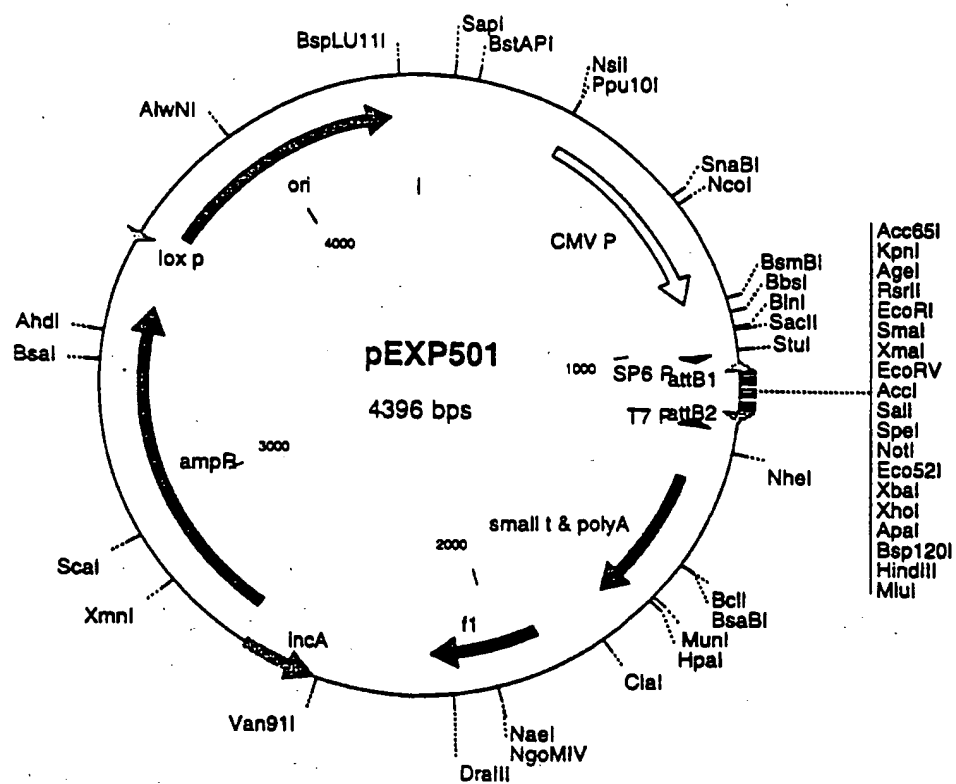
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2341 AAATGTCAGG CTCCCTTATA CACAGCCAGT CTGCAGGTCG ACCATAGTGA CTGGATATGT  
2401 TGTGTTTTTAC AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTTAA TATATGATA  
2461 TTTATATCAT TTTACGTTTC TCGTTCAGCT TTCTTGTAACA AAGTGGTTGA TCGCGTGCAT  
2521 GCGACGTCAT AGCTCTCTCC CTATAGTGAG TCGTATTATA AGCTAGGCAC TGGCCGTCGT  
2581 TTTACAACGT CGTGACTGGG AAAACTGCTA GCTTGGGATC TTTGTGAAGG AACCTTACTT  
2641 CTGTGGTGTG ACATAATGAG CAAACTACC TACAGAGATT TAAAGCTCTA AGGTAAATAT  
2701 AAAATTTTTTA AGTGTATAAT GTGTTAAACT AGCTGCATAT GCTTGCTGCT TGAGAGTTTT  
2761 GCTTACTGAG TATGATTAT GAAAATATTA TACACAGGAG CTAGTGATTG TAATTGTTTG  
2821 TGTATTTTATG ATTCACAGTC CCAAGGCTCA TTTGAGGCC CTCAGTCCTC ACAGTCTGTT  
2881 CATGATCATA ATCAGCCATA CCACATTTGT AGAGGTTTTA CTGCTTTAA AAAACCTCCC  
2941 ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT GTTGTGTTA ACTTGTTTAT  
3001 TGCAGCTTAT AATGGTTACA AATAAGCAA TAGCATCACA AATTTACAA ATAAAGCATT  
3061 TTTTTCACG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG  
3121 GATCGATCCT GCATTAATGA ATCGGCCAAC GCGCGGGAG AGGCGGTTG CGTATTGGCT  
3181 GGCGTAATAG CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG  
3241 GCGAATGGGA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA  
3301 GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCGCTCC TTTCGCTTC TTCCCTTCCT  
3361 TTCTCGCCAC GTTCGCGGCG TTTCCCGTC AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT  
3421 TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAACT TGATTAGGGT GATGGTTCAC  
3481 GTAGTGGGCC ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTCTT  
3541 TTAATAGTGG ACTCTTGTT CAACTGGA CAACACTCAA CCCTATCTG GTCTATTCTT  
3601 TTGATTTATA AGGGATTG CCGATTTCGG CCTATTGGTT AAAAAATGAG CTGATTTAAC  
3661 AAATATTTAA CGCGAATTT AACAAAATAT TAACGTTTAC AATTTGCGCT GATGCGGTAT  
3721 TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAC GCGGATCTGC GCAGCACCAT  
3781 GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC  
3841 AGCTGTGGAA TGTGTGTGAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGAGAA  
3901 GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC  
3961 CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC  
4021 TAACCTCGCC CATCCCGCCC CTAACCTCCG CCAGTTCCGC CCATTCTCCG CCCCCTGCT  
4081 GACTAATTTT TTTTATTAT GCAGAGGCCG AGGCGCGCTC GGCTCTGAG CTATTCCAGA  
4141 AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AAAGCTTGAT TCTTCTGACA  
4201 CAACAGTCTC GAACCTAAGG CTAGAGCCAC CATGATTGAA CAAGATGGAT TGCACGCAGG  
4261 TTCTCCGGCC GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC AGACAATCGG  
4321 CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG CGCCCGGTTT TTTTGTCAA  
4381 GACCGACCTG TCCGGTGCCC TGAATGAACT GCAGGACGAG GCAGCGCGGC TATCGTGGCT  
4441 GGCCACGACG GCGGTTCCCT GCGCAGCTGT GCTCGACGTT GCTACTGAAG CGGGAAGGGA  
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4681 CGGTCTTGTC GATCAGGATG ATCTGGACGA AGAGCATCAG GGGCTCGCGC CAGCCGAACT  
4741 GTTCGCCAGG CTCAAGGCCG GCATGCCCGA CGGCGAGGAT CTCGTCTGTA CCCATGGCGA  
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5101 TTTATTTTCA TTACATCTGT GTGTTGGTTT TTTGTGTGAA TCGATAGCGA TAAGGATCCG  
5161 CGTATGGTGC ACTCTCAGTA CAATCTGCTC TGATGCCGCA TAGTTAAGCC AGCCCCGACA  
5221 CCCGCCAACA CCCGCTGACG CGCCCTGACG GGCTTGTCTG CTCCCGGCAT CCGCTTACAG  
5281 ACAAGCTGTG ACCGCTCTCCG GGAGCTGCAT GTGTGAGAGG TTTTACCCTG CATCACCAGG  
5341 ACGCGCGAGA CGAAAGGGCC TCGTGATACG CCTATTTTAA TAGGTAAATG TCATGATAAT  
5401 AATGGTTTCT TAGACGTCAG GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTG  
5461 TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT  
5521 GCTTCAATAA TATTGAAAA GGAAGAGTAT GAGTATTCAA CATTTCCTG TCGCCCTTAT  
5581 TCCCTTTTTT GCGGCATTTT GCCTTCTCTG TTTTGTCTAC CCAGAAACGC TGGTGAAAGT  
5641 AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTAC ATCGAACTGG ATCTCAACAG  
5701 CGGTAAGATC CTTGAGAGTT TCGCCCCGA AGAACGTTTT CCAATGATGA GCACTTTTAA  
5761 AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTG

FIGURE 47C

5821 CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT  
5881 TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC  
5941 TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACC GCTTTTGTGA  
6001 CAACATGGGG GATCATGTAA CTCGCCCTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT  
6061 ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT TCGCAAACCT  
6121 ATTAAGTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC  
6181 GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA  
6241 TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CCGTATCATT GCAGCACTGG GGCCAGATGG  
6301 TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG  
6361 AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG CATTGGTAAC TGTCAGACCA  
6421 AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGTGATCTA  
6481 GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTCGTTCCA  
6541 CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG  
6601 CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA  
6661 TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAAA  
6721 TACTGTCTCT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC  
6781 TACATACCTC GCTCTGCTAA TCCTGTGTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG  
6841 TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT CGGGCTGAAC  
6901 GGGGGGTTTC TGCACACAGC CCAGCTTGGA GCGAACGACC TACACCGAAC TGAGATCACT  
6961 ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC  
7021 GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG  
7081 GTATCTTTAT AGTCCTGTCT GGTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG  
7141 CTCGTAGGGG GGGCGGAGCC TATGGAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCCT  
7201 GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCCTGCG TTATCCCCTG ATTCTGTGGA  
7261 TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA CGACCGAGCG  
7321 CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCCAATA CGCAAACCGC CTCTCCCCGC  
7381 GCGTTGGCCG ATTCATTAAT GCAGAGCTTG CAATTCGCGC GTTTTCAAT ATTATTGAAG  
7441 CATTTATCAG GGTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA  
7501 ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT  
7561 TATTATCATG ACATTAACCT ATAAAAATAG GCGTAGTACG AGGCCCTTTC ACTCATTAGA  
7621 TGCATGTCTG TACATAACTT ACGGTAAATG GCCCGCTGG CTGACCGCCC AACGACCCCC  
7681 GCCCATTGAC GTCAATAATG ACGTATGTTT CCATAGTAAC GCCAATAGGG ACTTTCCATT  
7741 GACGTCAATG GGTGGAGTAT TTACGGTAAA CTGCCCCACTT GGCAGTACAT CAAGTGTATC  
7801 ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGGTAA ATGGCCCGCC TGGCATTATG  
7861 CCCAGTACAT GACCTTATGG GACTTTCCTA CTTGGCAGTA CATCTACGTA TTAGTCATCG  
7921 CTATTACCAT GGTGATGCGG TTTTGGCAGT ACATCAATGG GCGTGGATAG CGGTTTGAAT  
7981 CACGGGGATT TCCAAGTCTC CACCCCATG ACGTCAATGG GAGTTTGTGTT TGGCACCAAA  
8041 ATCAACGGGA CTTTCCAAA TGTCGTAACA ACTCCGCCCC ATTGACGCAA ATGGGCGGTA  
8101 GCGGTGTACG GTGGGAGGTC TAT

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**Figure 4B A: pEXP501: pCMV.SPORT 6 host for attB Libraries**

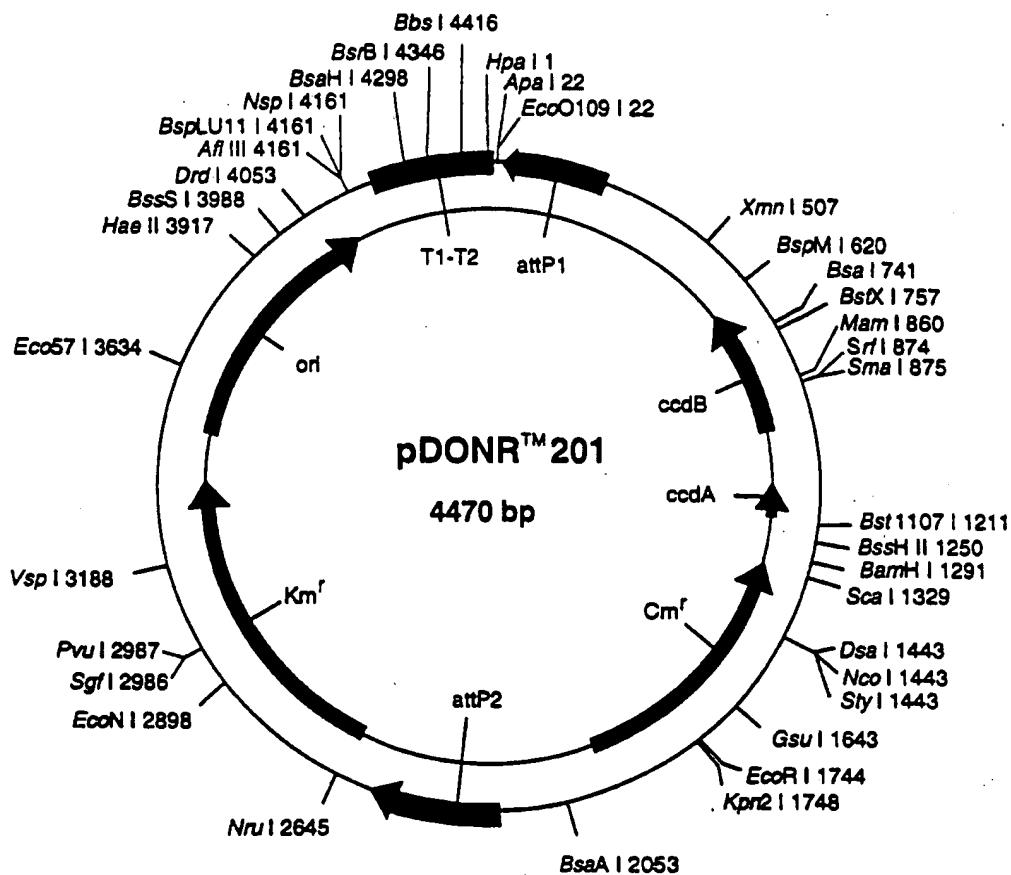




## pEXP501 4396 bp

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121 GATCGATCCA GACATGATAA GATACATTGA TGAGTTTGGA CAAACCACAA CTAGAATGCA  
181 GTGAAAAAAA TGCTTTATTT GTGAAATTTG TGATGCTATT GCTTTATTTG TAACCATTAT  
241 AAGCTGCAAT AAACAAGTTA ACAACAACAA TTGCATTCTAT TTTATGTTTC AGGTTTCAGGG  
301 GGAGGTGTGG GAGGTTTTTT AAAGCAAGTA AAACCTCTAC AAATGTGGTA TGGCTGATTA  
361 TGATCATGAA CAGACTGTGA GGACTGAGGG GCCTGAAATG AGCCTTGGGA CTGTGAATCT  
421 AAAATACACA AACAATTAGA ATCACTAGCT CCTGTGTATA ATATTTTCAT AAATCATACT  
481 CAGTAAGCAA AACTCTCAAG CAGCAAGCAT ATGCAGCTAG TTAAACACAT TATACACTTA  
541 AAAATTTTAT ATTTACCTTA GAGCTTTAAA TCTCTGTAGG TAGTTTGTCC AATTATGTCA  
601 CACCACAGAA GTAAGGTTCC TTCACAAAGA TCCCAAGCTA GCAGTTTTC CAGTCACGAC  
661 GTTGTA AAAAC GACGGCCAGT GCCTAGCTTA TAATACGACT CACTATAGGG ACCACTTTGT  
721 ACAAGAAAGC TGGGTACGCG TAAGCTTGGG CCCCTCGAGG GATCCTCTAG AGCGGCCGCG  
781 GACTAGTGAG CTCGTCGACG ATATCCCGGG AATCCCGGAC CGGTACCAGC CTGCTTTTTT  
841 GTACAACTT GTTCTATAGT GTCACCTAAA TAGGCCTAAT GGTCATAGCT GTTTCCTGTG  
901 TGAAATTGTT ATCCGCTCCG CGGCCTAGGC TAGAGTCCGG AGGCTGGATC GGTCCCGGTG  
961 TCTTCTATGG AGGTCAAAC AGCGTGGATG GCGTCTCCAG GCGATCTGAC GGTTCACATA  
1021 ACGAGCTCTG CTTATATAGA CCTCCACCG TACACGCCTA CCGCCCATTT CCGTCAATGG  
1081 GCGGAGTTG TTACGACATT TTGGAAAGTC CCGTTGATT TGGTGCCAAA ACAAACTCCC  
1141 ATTGACGTCA ATGGGGTGGA GACTTGGA AAA TCCCGTGAG TCAAACCGCT ATCCACGCCC  
1201 ATTGATGTAC TGCCAAAACC GCATCACCAT GGTAAATAGCG ATGACTAATA CGTAGATGTA  
1261 CTGCCAAGTA GGAAAGTCCC ATAAGGTCAT GTACTGGGCA TAATGCCAGG CGGGCCATTT  
1321 ACCGTCAATTG ACGTCAATAG GGGGCGTACT TGGCATATGA TACACTTGAT GTACTGCCAA  
1381 GTGGGCAGTT TACCGTAAAT ACTCCACCCA TTGACGTCAA TGGAAAGTCC CTATTGGCGT  
1441 TACTATGGGA ACATACGTCA TTATTGACGT CAATGGGCGG GGGTCGTTGG GCGGTCAGCC  
1501 AGGCGGGCCA TTTACCGTAA GTTATGTAAAC GACATGCATC TAATGAGTGA AAGGGCCTCG  
1561 TACTACGCCT ATTTTATAG GTTAATGTCA TGATAATAAT GGTTTCTTAG ACGTCAGGTG  
1621 GCACTTTTCG GGGAAATGTG CGCGGAACCC CTATTTGTTT ATTTTCTAA ATACATTCAA  
1681 ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT TCAATAATAT TGAAAAACGC  
1741 GCGAATTGCA AGCTCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC GGTTTGCGTA  
1801 TTGGGCGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CCGCTGCGGC  
1861 GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG  
1921 CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT  
1981 TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA TCACAAAAAT CGACGCTCAA  
2041 GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA GCGGTTTCCC CCTGGAAGCT  
2101 CCCTCGTGCG CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC  
2161 CTTGCGGAAG CGTGGCGCTT TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG  
2221 TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT  
2281 TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA CGACTTATCG CCACTGGCAG  
2341 CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA  
2401 AGTGGTGGCC TAACACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA  
2461 AGCCAGTTAC CTTGCGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAAACA ACCACCGCTG  
2521 GTAGCGGTGG TTTTTTGTG TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG  
2581 AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG GAACGAAAAAC TCACGTAAAG  
2641 GGATTTTGGT CATGCCATAA CTTGCTATAG CATAATTAT ACGAAGTTAT GGCATGAGAT  
2701 TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTT AAATCAATCT  
2761 AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA  
2821 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC GTGTAGATAA  
2881 CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC  
2941 GCTCACCGGC TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA  
3001 GTGGTCTGCG AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG  
3061 TAAGTAGTTC GCCAGTTAAT AGTTTGCAGC ACGTTGTTGC CATTGCTACA GGCATCGTGG  
3121 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA TCAAGGCGAG-

3181 TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTTCCGGTCCT CCGATCGTTG  
3241 TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC  
3301 TTAGTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT  
3361 TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC GCGGTCAATA CGGGATAATA  
3421 CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT TCGGGGCGAA  
3481 AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA  
3541 ACTGATCTTC AGCATCTTTT ACTTTACCCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC  
3601 AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAATG TTGAATACTC ATACTCTTCC  
3661 TTTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGCCAGGG GTGGGCACAC  
3721 ATATTTGATA CCAGCGATCC CTACACAGCA CATAATTCAA TGCGACTTCC CTCTATCGCA  
3781 CATCTTAGAC CTTTATTCTC CCTCCAGCAC ACATCGAAGC TGCCGAGCAA GCCGTTCTCA  
3841 CCAGTCCAAG ACCTGGCATG AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA  
3901 TAGGGGTTC GCGCACATTT CCCCAGAAAAG TGCCACCTGA AATTGTAAAC GTTAATATTT  
3961 TGTTAAAATT CGCGTTAAAT TTTTGTTAAA TCAGCTCATT TTTTAACCAA TAGGCCGAAA  
4021 TCGGCAAAAT CCCTTATAAA TCAAAAGAAT AGACCGAGAT AGGGTTGAGT GTTGTTCCAG  
4081 TTTGGAACAA GAGTCCACTA TTAAAGAACG TGGACTCCAA CGTCAAAGGG CGAAAAACCG  
4141 TCTATCAGGG CGATGGCCCA CTACGTGAAC CATCACCTTA ATCAAGTTTT TTGGGGTCGA  
4201 GGTGCCGTAA AGCACTAAAT CGGAACCCTA AAGGGAGCCC CCGATTAGA GCTTGACGGG  
4261 GAAAGCCGCG GAACGTGGCG AGAAAGGAAG GGAAGAAAGC GAAAGGAGCG GGCCTAGGG  
4321 CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG TAACCACCAC ACCCGCCGCG CTTAATGCGC  
4381 CGCTACAGGG CGCGTC



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## pDONR201 4470 bp (rotated to position 3516)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
260..29		attP1
656..961		ccdB
1099..1184		ccdA
1303..1962		CmR
2210..2442		attP2
2565..3374		Kmr
3495..4134		ori
1	GTTAACGCTA GCATGGATCT CGGGCCCCAA ATAATGATTT TATTTTGACT GATAGTGACC	
61	TGTTCTGTTGC AACAAATTGA TGAGCAATGC TTTTATATAA TGCCAACCTT GTACAAAAA	
121	GCTGAACGAG AAACGTAAAA TGATATAAAT ATCAATATAT TAAATTAGAT TTGTCATAAA	
181	AAACAGACTA CATAATACTG TAAAAACAAA CATATCCAGT CACTATGAAT CAACTACTTA	
241	GATGGTATTA GTGACCTGTA GTCGACCGAC AGCCTTCCAA ATGTTCTTCG GGTGATGCTG	
301	CCAACTTAGT CGACCGACAG CCTTCCAAAT GTTCTTCTCA AACGGAATCG TCGTATCCAG	
361	CCTACTCGCT ATTGTCTCTCA ATGCCGTATT AAATCATAAA AAGAAATAAG AAAAAGAGGT	
421	GCGAGCCTCT TTTTGTGTG ACAAATAAAA AACATCTACC TATTCATATA CGCTAGTGTG	
481	ATAGTCTCTG AAATCATCTG CATCAAGAAC AATTCACAA CTCTTATACT TTTCTCTTAC	
541	AAGTCGTTCT GCTTCATCTG GATTTTCAGC CTCTATACTT ACTAAACGTG ATAAAGTTTC	
601	TGTAATTTCT ACTGTATCGA CCGTGCAGCT GGCTGTGTAT AAGGGAGCCT GACATTTATA	
661	TTCCCCAGAA CATCAGGTTA ATGGCGTTTT TGATGTCATT TTCGCGGTGG CTGAGATCAG	
721	CCACTTCTTC CCCGATAACG GAGACCGGCA CACTGGCCAT ATCGGTGGTC ATCATGCGCC	
781	AGCTTTCATC CCCGATATGC ACCACCGGGT AAAGTTCAGG GGAGACTTTA TCTGACAGCA	
841	GACGTGCACT GGCCAGGGGG ATCACCATCC GTCGCCCGGG CGTGCAATA ATATCACTCT	
901	GTACATCCAC AAACAGACGA TAACGGCTCT CTCTTTTATA GGTGTAAACC TTAAACTGCA	
961	TTTCACCAGT CCCTGTTCTC GTCAGCAAAA GAGCCGTTCA TTTCAATAAA CCGGGCGACC	
1021	TCAGCCATCC CTTCCTGATT TTCCGCTTTC CAGCGTTCGG CACGCAGACG ACGGGCTTCA	
1081	TTCTGCATGG TTGTGCTTAC CAGACCGGAG ATATTGACAT CATATATGCC TTGAGCAACT	
1141	GATAGCTGTC GCTGTCAACT GTCAGTGTAA TACGCTGCTT CATAGCACAC CTCTTTTGA	
1201	CATACTTCGG GTATACATAT CAGTATATAT TCTTATACCG CAAAAATCAG CGCGCAAATA	
1261	CGCATACTGT TATCTGGCTT TTAGTAAGCC GGATCCACGC GATTACGCCC CGCCTGCCA	
1321	CTCATCGCAG TACTGTTGTA ATTCATTAAAG CATTCTGCCG ACATGGAAGC CATCACAGAC	
1381	GGCATGATGA ACCTGAATCG CCAGCGGCAT CAGCACCTTG TCGCCTTGGC TATAATATT	
1441	GCCCATGGTG AAAACGGGGG CGAAGAAGTT GTCCATATTG GCCACGTTTA AATCAAAACT	
1501	GGTGAAACTC ACCCAGGGAT TGGCTGAGAC GAAAAACATA TTCTCAATAA ACCCTTTAGG	
1561	GAAATAGGCC AGGTTTTCAC CGTAACACGC CACATCTTGC GAATATATGT GTAGAAACTG	
1621	CCGGAATCG TCGTGGTATT CACTCCAGAG CGATGAAAAC GTTTCAGTTT GCTCATGGAA	
1681	AACGGTGTAA CAAGGGTGAA CACTATCCCA TATCACCAGC TCACCGTCTT TCATTGCCAT	
1741	ACGGAATTCC GGATGAGCAT TCATCAGGCG GGCAAGAATG TGAATAAAGG CCGGATAAAA	
1801	CTTGTGCTTA TTTTCTTTA CGGTCTTTAA AAAGGCCGTA ATATCCAGCT GAACGGTCTG	
1861	GTTATAGGTA CATTGAGCAA CTGACTGAAA TGCCTCAAAA TGTTCTTTAC GATGCCATTG	
1921	GGATATATCA ACGGTGGTAT ATCCAGTGAT TTTTCTCTCC ATTTTAGCTT CCTTAGCTCC	
1981	TGAAATCTC GATAACTCAA AAAATACGCC CGGTAGTGAT CTTATTTCAT TATGGTGAAA	
2041	GTTGGAACCT CTTACGTGCC GATCAACGTC TCATTTTCGC CAAAAGTTGG CCCAGGGCTT	
2101	CCCGGTATCA ACAGGGACAC CAGGATTTAT TTATTCTGCG AAGTGATCTT CCGTCACAGG	
2161	TATTTATTTC GCGCAAAGTG CGTCGGGTGA TGCTGCCAAC TTAGTCGACT ACAGGTCACT	
2221	AATACCATCT AAGTAGTTGA TTCATAGTGA CTGGATATGT TGTGTTTTAC AGTATTATGT	
2281	AGTCTGTTTT TTATGCAAAA TCTAATTTAA TATATTGATA TTTATATCAT TTTACGTTTC	
2341	TCGTTCACTT TTCTGTGACA AAGTTGGCAT TATAAGAAAG CATTGCTTAT CAATTTGTTG	
2401	CAACGAACAG GTCACATCA GTCAAAATAA AATCATTATT TGCCATCCAG CTGCAGCTCT	
2461	GGCCCGTGTG TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA TCATCATGAA	
2521	CAATAAAACT GTCTGCTTAC ATAAACAGTA ATACAAGGGG TGTTATGAGC CATATTCAAC	
2581	GGGAAACGTC GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTTATAT GGGTATAAAT	
2641	GGGCTCGCGA TAATGTCGGG CAATCAGGTG CGACAATCTA TCGCTTGTAT GGGGAAGCCCG	
2701	ATGCGCCAGA GTTGTCTCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT GTTACAGATG -	

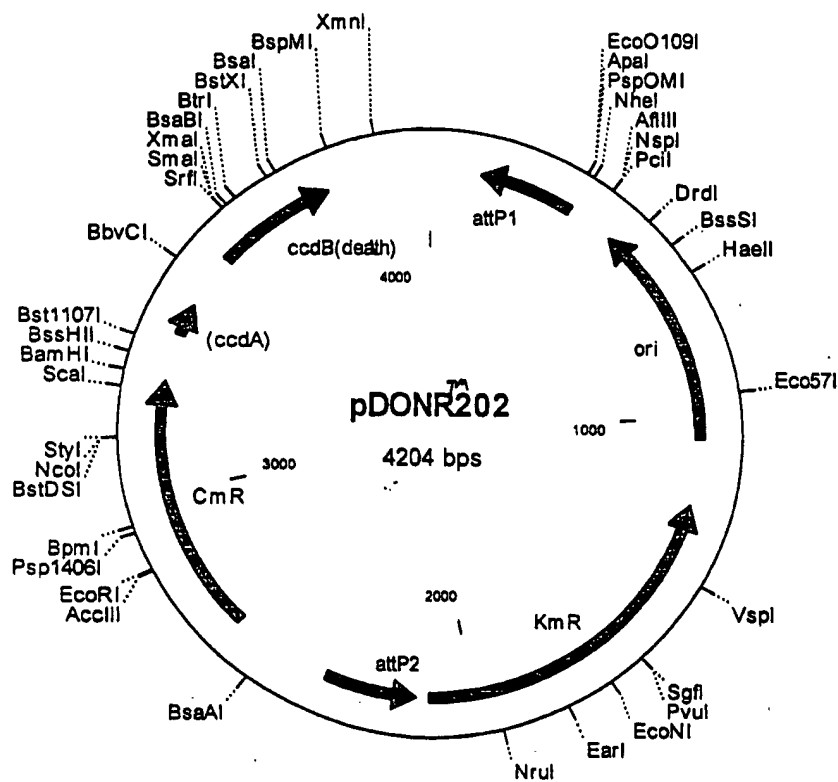
FIGURE 49B

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2761 AGATGGTCAG ACTAAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC AAGCATTTTA  
2821 TCCGTACTCC TGATGATGCA TGGTTACTCA CCACTGCGAT CCCCGGAAAA ACAGCATTC  
2881 AGGTATTAGA AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG GCAGTGTTC  
2941 TCGCCCGGTT GCATTTCGATT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT CGCGTATTT  
3001 GTCTCGCTCA GGCAGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT GATTTTGATG  
3061 ACGAGCGTAA TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATAAA CTTTGTCCAT  
3121 TCTACCCGGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT ATTTTGTACG  
3181 AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC CGATACCAGG  
3241 ATCTTGCCAT CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG AAACGGCTTT  
3301 TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT TTGATGCTCG  
3361 ATGAGTTTTT CTAATCAGAA TTGGTTAATT GGTGTAAACA CTGGCAGAGC ATTACGCTGA  
3421 CTTGACGGGA CGGCGCAAGC TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG  
3481 AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT  
3541 AATCTGCTGC TTGCAAACAA AAAAACCCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA  
3601 AGAGCTACCA ACTCTTTTTT CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC  
3661 TGTCCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC  
3721 ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT  
3781 TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG  
3841 GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC ACCGAAGTGA GATACCTACA  
3901 GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT  
3961 AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA  
4021 TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC  
4081 GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG GCCTTTTTAC GGTTCCTGGC  
4141 CTTTTGCTGG CTTTTTGCTC ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA  
4201 CCGTATTACC GCTAGCCAGG AAGAGTTTGT AGAAACGCAA AAAGGCCATC CGTCAGGATG  
4261 GCCTTCTGCT TAGTTTGATG CCTGGCAGTT TATGGCGGGC GTCTGCCCCG CCACCTCCG  
4321 GGCCGTTGCT TCACAACGTT CAAATCCGCT CCCGGCGGAT TTGTCTACT CAGGAGAGCG  
4381 TTCACCGACA AACAACAGAT AAAACGAAAG GCCCAGTCTT CCGACTGAGC CTTTCGTTTT  
4441 ATTTGATGCC TGGCAGTTCC CTACTCTCGC

FIGURE 49C

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FIGURE 50A: pDONR202 (kan<sup>R</sup>)



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## pDONR202 4204 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>				
369..127		attP1				
486..1059		ori				
1228..2107		KmR				
2381..2140		attP2				
2629..3288		CmR				
3408..3492		inactivated ccdA				
3630..3935		ccdB				
1	CGGCATTGAG	GACAATAGCG	AGTAGGCTGG	ATACGACGAT	TCCGTTTGAG	AAGAACATTT
61	GGAAGGCTGT	CGGTCGACTA	AGTTGGCAGC	ATCACCCGAA	GAACATTTGG	AAGGCTGTCTG
121	GTCGACTACA	GGTCACTAAT	ACCATCTAAG	TAGTTGATTC	ATAGTGACTG	GATATGTTGT
181	GTTTTACAGT	ATTATGTAGT	CTGTTTTTTA	TGCAAAATCT	AATTTAATAT	ATTGATATTT
241	ATATCATTTT	ACGTTTCTCG	TTCAGCTTTT	TTGTACAAAG	TTGGCATTAT	AAAAAAGCAT
301	TGCTCATCAA	TTTGTGCAA	CGAACAGGTC	ACTATCAGTC	AAAATAAAAT	CATTATTTGG
361	GGCCCCGAGAT	CCATGCTAGC	GGTAATACGG	TTATCCACAG	AATCAGGGGA	TAACGCAGGA
421	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG
481	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG
541	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC
601	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCGCGCTT	TCTCCCTTCG
661	GGAAGCGTGG	CGCTTTCTCA	TAGCTCACGC	TGTAGGTATC	TCAGTTCCGT	GTAGGTCGTT
721	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAAG	CCGACCGCTG	CGCCTTATCC
781	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT	GGCAGCAGCC
841	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG
901	TGGCCTAACT	ACGCTACAC	TAGAAGGACA	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA
961	GTTACCTTCG	GA AAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC
1021	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT
1081	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAAACG	AAAACTCACG	TTAAGGGATT
1141	TTGTCATGA	GCTTGCGCCG	TCCCGTCAAG	TCAGCGTAAT	GCTCTGCCAG	TGTTACAACC
1201	AATTAACCAA	TTCTGATTAG	AAAAACTCAT	CGAGCATCAA	ATGAAACTGC	AATTTATTCA
1261	TATCAGGATT	ATCAATACCA	TATTTTTGAA	AAAGCCGTTT	CTGTAATGAA	GGAGAAAAC
1321	CACCGAGGCA	GTTCCATAGG	ATGGCAAGAT	CCTGGTATCG	GTCTGCGATT	CCGACTCGTC
1381	CAACATCAAT	ACAACCTATT	AATTTCCCCT	CGTCAAAAAT	AAGGTTATCA	AGTGAGAAAT
1441	CACCATGAGT	GACGACTGAA	TCCGGTGAGA	ATGGCAAAAG	TTTATGCATT	TCTTTCCAGA
1501	CTTGTTCAAC	AGGCCAGCCA	TTACGCTCGT	CATCAAAATC	ACTCGCATCA	ACCAAACCGT
1561	TATTCATTTC	TGATTGCGCC	TGAGCGAGAC	GAAATACGCG	ATCGCTGTGA	AAAGGACAAT
1621	TACAAACAGG	AATCGAATGC	AACCGCGCCA	GGAACACTGC	CAGCGCATCA	ACAATATTTT
1681	CACCTGAATC	AGGATATTCT	TCTAATACCT	GGAATGCTGT	TTTCCGGGG	ATCGCAGTGG
1741	TGAGTAACCA	TGCATCATCA	GGAGTACGGA	TAAATGCTT	GATGGTCGGA	AGAGGCATAA
1801	ATTCGGTCAG	CCAGTTTAGT	CTGACCATCT	CATCTGTAAC	ATCATTGGCA	ACGCTACCTT
1861	TGCCATGTTT	CAGAAACAAC	TCTGGCGCAT	CGGGCTTCCC	ATACAAGCGA	TAGATTGTCTG
1921	CACCTGATTG	CCCGACATTA	TCGCGAGCCC	ATTTATACCC	ATATAAATCA	GCATCCATGT
1981	TGGAATTTAA	TCGCGGCCCTC	GACGTTTCCC	GTTGAATATG	GCTCATAACA	CCCCTTGTAT
2041	TACTGTTTAT	GTAAGCAGAC	AGTTTTATTG	TTCATGATGA	TATATTTTTA	TCTTGTGCAA
2101	TGTAACATCA	GAGATTTTGA	GACACGGGCC	AGAGCTGCAG	CTGGATGGCA	AATAATGATT
2161	TTATTTTGAC	TGATAGTGAC	CTGTTCTGTTG	CAACAAATG	ATAAGCAATG	CTTTCTTATA
2221	ATGCCAACTT	TGTACAAGAA	AGCTGAACGA	GAAACGTAAA	ATGATATAAA	TATCAATATA
2281	TTAAATTAGA	TTTTCATATA	AAAACAGACT	ACATAATACT	GTAAAACACA	ACATATCCAG
2341	TCACATGAA	TCAACTACTT	AGATGGTATT	AGTGACCTGT	AGTCGACTAA	GTTGGCAGCA
2401	TCACCCGACG	CACTTTGCGC	CGAATAAATA	CCTGTGACGG	AAGATCACTT	CGCAGAATAA
2461	ATAAATCCTG	GTGTCCTGT	TGATACCGGG	AAGCCCTGGG	CCAACCTTTG	GCGAAAATGA
2521	GACGTTGATC	GGCACGTAAG	AGGTTCCAAC	TTTCACCATA	ATGAAATAAG	ATCACTACCG
2581	GGCGTATTTT	TTGAGTTATC	GAGATTTTCA	GGAGCTAAGG	AAGCTAAAAT	GGAGAAAAAA
2641	ATCACTGGAT	ATACCACCGT	TGATATATCC	CAATGGCATC	GTAAAGAACA	TTTTGAGGCA
2701	TTTCAGTCAG	TTGCTCAATG	TACCTATAAC	CAGACCGTTC	AGCTGGATAT	TACGGCCTTT -

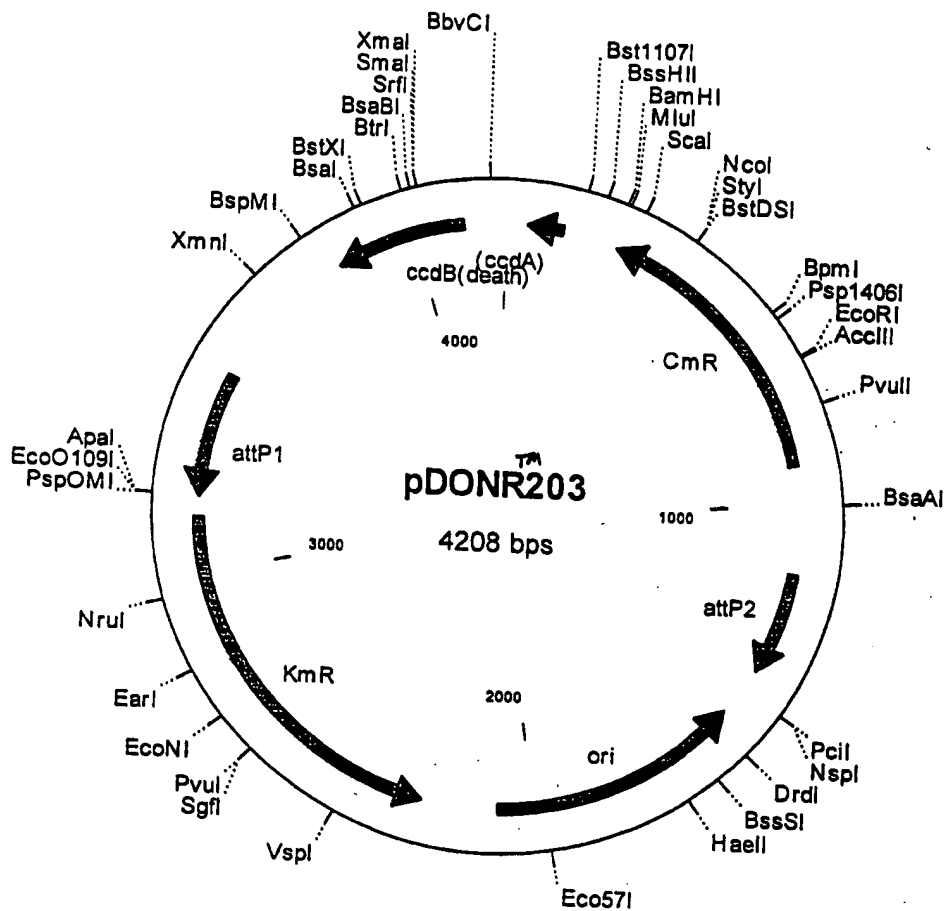
FIGURE 50B



2761 TTAAAGACCG TAAAGAAAA TAAGCACAAG TTTTATCCGG CCTTTATTCA CATTCTTGCC  
2821 CGCCTGATGA ATGCTCATCC GGAATTCGGT ATGGCAATGA AAGACGGTGA GCTGGTGATA  
2881 TGGGATAGTG TTCACCCTTG TTACACCGTT TTCCATGAGC AAAGTGAAC GTTTTCATCG  
2941 CTCTGGAGTG AATACCACGA CGATTTCCGG CAGTTTCTAC ACATATATTC GCAAGATGTG  
3001 GCGTGTTACG GTGAAAACCT GGCCTATTTT CCTAAAGGGT TTATTGAGAA TATGTTTTTC  
3061 GTCTCAGCCA ATCCCTGGGT GAGTTTCACC AGTTTTGATT TAAACGTGGC CAATATGGAC  
3121 AACTTCTTCG CCCCCGTTTT CACCATGGGC AAATATTATA CGCAAGGCGA CAAGGTGCTG  
3181 ATGCCGCTGG CGATTCAGGT TCATCATGCC GTCTGTGATG GCTTCCATGT CGGCAGAATG  
3241 CTTAATGAAT TACAACAGTA CTGCGATGAG TGGCAGGGCG GGGCGTAATC GCGTGGATCC  
3301 GGCTTACTAA AAGCCAGATA ACAGTATGCG TATTTGCGCG CTGATTTTGT CGGTATAAGA  
3361 ATATATACTG ATATGTATAC CCGAAGTATG TCAAAAAGAG GTGTGCTATG AAGCAGCGTA  
3421 TTACAGTGAC AGTTGACAGC GACAGCTATC AGTTGCTCAA GGCATATATG ATGTCAATAT  
3481 CTCCGGTCTG GTAAGCAGAA CCATGCAGAA TGAAGCCCGT CGTCTGCGTG CCGAACGCTG  
3541 GAAAGCGGAA AATCAGGAAG GGATGGCTGA GGTGCGCCGG TTTATTGAAA TGAACGGCTC  
3601 TTTTGCTGAC GAGAACAGGG ACTGGTGAAA TGCAGTTTAA GGTTTACACC TATAAAGAG  
3661 AGAGCCGTTA TCGTCTGTTT GTGGATGTAC AGAGTGATAT TATTGACACG CCCGGGCGAC  
3721 GGATGGTGAT CCCCCTGGCC AGTGCACGTC TGCTGTCAGA TAAAGTCTCC CGTGAACTTT  
3781 ACCCGGTGGT GCATATCGGG GATGAAAGCT GCGCGATGAT GACCACCGAT ATGGCCAGTG  
3841 TGCCGGTCTC CGTTATCGGG GAAGAAGTGG CTGATCTCAG CCACCGCGAA AATGACATCA  
3901 AAAACGCCAT TAACCTGATG TTCTGGGGAA TATAAATGTC AGGCTCCCTT ATACACAGCC  
3961 AGTCTGCAGG TCGATACAGT AGAAATTACA GAAACTTTAT CACGTTTAGT AAGTATAGAG  
4021 GCTGAAAATC CAGATGAAGC CGAACGACTT GTAAGAGAAA AGTATAAGAG TTGTGAAATT  
4081 GTTCTTGATG CAGATGATTT TCAGGACTAT GACACTAGCG TATATGAATA GGTAGATGTT  
4141 TTTATTTTGT CACACAAAA AGAGGCTCGC ACCTCTTTTT CTTATTTCTT TTTATGATTT  
4201 AATA

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FIGURE 51A pDONR203 (kan<sup>R</sup>)



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## pDONR203 4208 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
47..131	inactivated ccdA
251..910	CmR
1158..1398	attP2
1509..2082	ori
2251..3130	KmR
3464..3174	attP1
3812..4117	ccdB

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1 GCGTTCGGCA CGCAGACGAC GGGCTTCATT CTGCATGGTT GTGCTTACCA GACCCGAGAT
61 ATTGACATCA TATATGCCTT GAGCAACTGA TAGCTGTCGC TGTCAACTGT CACTGTAATA
121 CGCTGCTTCA TAGCACACCT CTTTTTGACA TACTTCGGGT ATACATATCA GTATATATTC
181 TTATACCGCA AAAATCAGCG CGCAAATACG CATACTGTTA TCTGGCTTTT AGTAAGCCGG
241 ATCCACGCGT TTACGCCCGG CCCTGCCACT CATCGCAGTA CTGTTGTAAT TCATTAAGCA
301 TTCTGCCGAC ATGGAAGCCA TCACAGACGG CATGATGAAC CTGAATCGCC AGCGGCATCA
361 GCACCTTGTC GCCTTGCGTA TAATATTTGC CCATGGTGAA AACGGGGGCG AAGAAGTTGT
421 CCATATTGGC CACGTTTAAA TCAAACTGG TGAACTCAC CCAGGGATTG GCTGAGACGA
481 AAAACATATT CTCAATAAAC CCTTTAGGGA AATAGGCCAG GTTTTCACCG TAACACGCCA
541 CATCTTGCGA ATATATGTGT AGAACTGCC GGAAATCGTC GTGGTATTCA CTCCAGAGCG
601 ATGAAAACGT TTCAGTTTGC TCATGAAAAA CGGTGTAACA AGGGTGAACA CTATCCATA
661 TCACCAGCTC ACCGTCTTTC ATTGCCATAC GGAATTCGGG ATGAGCATTC ATCAGGCGGG
721 CAAGAATGTG AATAAAGGCC GGATAAAACT TGTGCTTATT TTTCTTTACG GTCTTTAAAA
781 AGGCCGTAAT ATCCAGCTGA ACGGTCTGGT TATAGGTACA TTGAGCAACT GACTGAAATG
841 CCTCAAAATG TTCTTTACGA TGCCATTGGG ATATATCAAC GGTGGTATAT CCAGTGATTT
901 TTTTCTCCAT TTTAGCTTCC TTAGCTCCTG AAAATCTCGA TAACTCAAAA AATACGCCCG
961 GTAGTGATCT TATTTTCATTA TGGTGAAAGT TGGAACCTCT TACGTGCCGA TCAACGTCTC
1021 ATTTTCGCCA AAAGTTGGCC CAGGGCTTCC CGGTATCAAC AGGGACACCA GGATTTATTT
1081 ATTCTGCGAA GTGATCTTCC GTCACAGGTA TTTATTCGGC GCAAAGTGCG TCGGGTGATG
1141 CTGCCAACTT AGTCGACTAC AGGTCACTAA TACCATCTAA GTAGTTGATT CATAGTGACT
1201 GGATATGTTG TGTTTTACAG TATTATGTAG TCTGTTTTTT ATGCAAAATC TAATTTAATA
1261 TATTGATATT TATATCATT TACGTTTCTC GTTCAGCTTT CTTGTACAAA GTTGGCATT
1321 TAAGAAAGCA TTGCTTATCA ATTTGTTGCA ACGAACAGGT CACTATCAGT CAAAATAAAA
1381 TCATTATTTG CCATCCAGCT AGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA
1441 GGAAGAACA TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG
1501 CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT
1561 CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC
1621 CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT
1681 TCGGGAAGCG TGGCGCTTTC TCATAGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC
1741 GTTCGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCCGTTC AGCCCGACCG CTGCGCCTTA
1801 TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA
1861 GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG
1921 TGGTGGCCTA ACTACGGCTA CACTAGAAGA ACAGTATTTG GTATCTGCGC TCTGCTGAAG
1981 CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT
2041 AGCGGTGGTT TTTTGTGTTG CAAGCAGCAG ATTACGCGCA GAAAAAAGG ATCTCAAGAA
2101 GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG
2161 ATTTTGGTCA TGAGCTTGCG CCGTCCCGTC AAGTCAGCGT AATGCTCTGC CAGTGTTACA
2221 ACCAATTAAC CAATTCTGAT TAGAAAACT CATCGAGCAT CAAATGAAAC TGCAATTTAT
2281 TCATATCAGG ATTATCAATA CCATATTTT GAAAAAGCCG TTTCTGTAAT GAAGGAGAAA
2341 ACTCACCGAG GCAGTTCCAT AGGATGGCAA GATCCTGGTA TCGGTCTGCG ATTCCGACTC
2401 GTCCAACATC AATACAACCT ATTAATTTCC CCTCGTCAAA AATAAGGTTA TCAAGTGAGA
2461 AATCACCATG AGTGACGACT GAATCCGGTG AGAATGGCAA AAGTTTATGC ATTTCTTTCC
2521 AGACTTGTTT AACAGGCCAG CCATTACGCT CGTCATCAAA ATCACTCGCA TCAACCAAAC
2581 CGTTATTTCAT TCGTGATTGC GCCTGAGCGA GACGAAATAC GCGATCGCTG TTAAGAGGAC
2641 AATTACAAAC AGGAATCGAA TGCAACCGGC GCAGGAACAC TGCCAGCGCA TCAACAATAT
2701 TTTACCTGA ATCAGGATAT TCTTCTAATA CCTGGAATGC TGTTTTTCCG GGGATCGCAG-

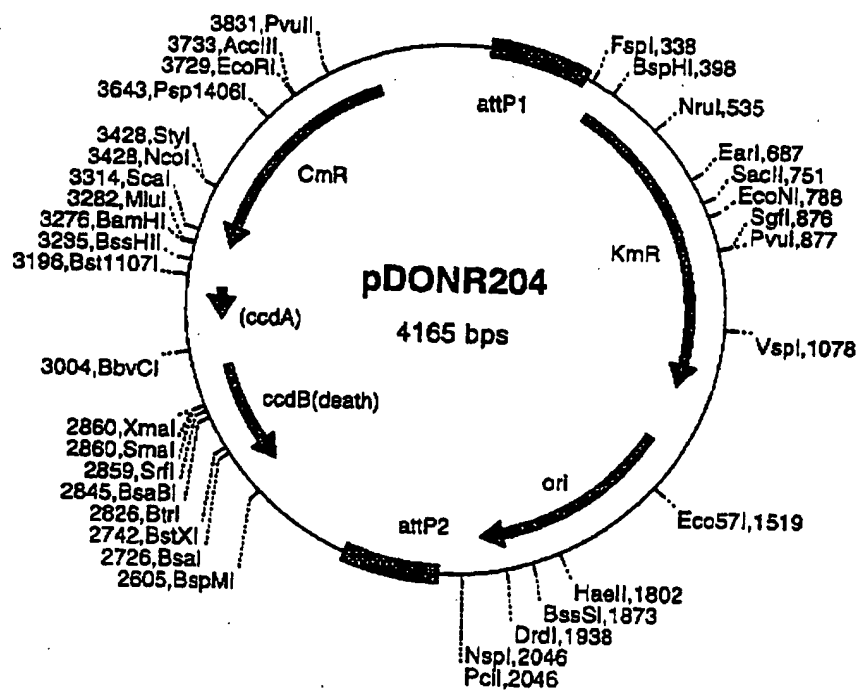
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FIGURE 51B

2761 TGGTGAGTAA CCATGCATCA TCAGGAGTAC GGATAAAATG CTTGATGGTC GGAAGAGGCA  
2821 TAAATTCCGT CAGCCAGTTT AGTCTGACCA TCTCATCTGT AACATCATTG GCAACGCTAC  
2881 CTTTGCCATG TTTCAGAAAC AACTCTGGCG CATCGGGCTT CCCATACAAG CGATAGATTG  
2941 TCGCACCTGA TTGCCCCGACA TTATCGCGAG CCCATTTATA CCCATATAAA TCAGCATCCA  
3001 TGTTGGAATT TAATCGCGGC CTCGACGTTT CCCGTTGAAT ATGGCTCATA ACACCCCTTG  
3061 TATTACTGTT TATGTAAGCA GACAGTTTTA TTGTTTCATGA TGATATATTT TTATCTTGTTG  
3121 CAATGTAACA TCAGAGATTT TGAGACACGG GCCAGAGCTG CAGCTAGCAT GGATCTCGGG  
3181 CCCCAAATAA TGATTTTATT TTGACTGATA GTGACCTGTT CGTTGCAACA AATTGATGAG  
3241 CAATGCTTTT TTATAATGCC AACTTTGTAC AAAAAAGCTG AACGAGAAAC GTAAAATGAT  
3301 ATAAATATCA ATATATTAAA TTAGATTTTG CATAAAAAAC AGACTACATA ATACTGTAAA  
3361 ACACAACATA TCCAGTCACT ATGAATCAAC TACTTAGATG GTATTAGTGA CCTGTAGTCG  
3421 ACCGACAGCC TTCCAAATGT TCTTCGGGTG ATGCTGCCAA CTTAGTCGAC CGACAGCCTT  
3481 CCAAATGTTT TTCTCAAACG GAATCGTCGT ATCCAGCCTA CTCGCTATTG TCCTCAATGC  
3541 CGTATTAAAT CATAAAAAGA AATAAGAAAA AGAGGTGCGA GCCTCTTTTT TGTGTGACAA  
3601 AATAAAAACA TCTACCTATT CATATACGCT AGTGTCATAG TCCTGAAAAT CATCTGCATC  
3661 AAGAACAATT TCACAACCTT TATACTTTTC TCTTACAAGT CGTTCGGCTT CATCTGGATT  
3721 TTCAGCCTCT ATACTTACTA AACGTGATAA AGTTTCTGTA ATTTCTACTG TATCGACCTG  
3781 CAGACTGGCT GTGTATAAGG GAGCCTGACA TTTATATTCC CCAGAACATC AGGTTAATGG  
3841 CGTTTTTGAT GTCATTTTCG CGGTGGCTGA GATCAGCCAC TTCTTCCCCG ATAACGGAGA  
3901 CCGGCACACT GGCCATATCG GTGGTCATCA TGCGCCAGCT TTCATCCCCG ATATGCACCA  
3961 CCGGGTAAAG TTCACGGGAG ACTTTATCTG ACAGCAGACG TGCCTGGCC AGGGGGATCA  
4021 CCATCCGTCG CCCGGGCGTG TCAATAATAT CACTCTGTAC ATCCACAAAC AGACGATAAC  
4081 GGCTCTCTCT TTTATAGGTG TAAACCTTAA ACTGCATTTC ACCAGTCCCT GTTCTCGTCA  
4141 GCAAAAAGAG CGTTCATTTC AATAAACCGG GCGACCTCAG CCATCCCTTC CTGATTTTCC  
4201 GCTTTCCA

FIGURE 51C

FIGURE 52A pDONR204 (kan<sup>R</sup>)



## pDONR204 4165 bp

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1 CGGCATTGAG GACAATAGCG AGTAGGCTGG ATACGACGAT TCCGTTTGAG AAGAACATTT
61 GGAAGGCTGT CGGTCGACTA CAGGTCACTA ATACCATCTA AGTAGTTGAA TCATAGTGAC
121 TGGATATGTT GTGTTTTACA GTATTATGTA GTCTGTTTTT TATGCAAAAT CTAATTTAAT
181 ATATTGATAT TTATATCATT TTACGTTTCT CGTTCAGCTT TTTTGTACAA AGTTGGCATT
241 ATAAAAAGC ATTGCTTATC AATTTGTGTC AACGAACAGG TCACTATCAG TCAAAATAAA
301 ATCATTTATT GGGGCCCGAG ATCCATGCGT GCTGCAGTGC GCAGGGCCCCG TGTCTCAAAA
361 TCTCTGATGT TACATTGCAC AAGATAAAAA TATATCATCA TGAACAATAA AACTGTCTGC
421 TTACATAAAC AGTAATACAA GGGGTGTTAT GAGCCATATT CAACGGGAAA CGTCTTGCTG
481 GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTATATAT GGGTATAAAT GGGCTCGCGA
541 TAATGTCGGG CAATCAGGTG CGACAATCTT TCGATTGTAT GGGGAAGCCCG ATGCGCCAGA
601 GTTGTCTCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT GTTACAGATG AGATGGTCAG
661 ACTAAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC AAGCATTTTA TCCGTACTCC
721 TGTGATGCA TGGTTACTCA CCACTGCGAT CCGCGGGAAA ACAGCATTCC AGGTATTAGA
781 AGAATATCCT GATTGAGGTG AAAATATTGT TGATGCGCTG GCAGTGTTCG TGCGCCGGTT
841 GCATTGCGATT CCTGTTTGTA ATTGTCTCTT TAACAGCGAT CGCGTATTTT GTCTCGCTCA
901 GGCAGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT GATTTTGATG ACGAGCGTAA
961 TGGCTGGCCT GTTGAACAAG TCTGGAAAAG AATGCATACG CTTTGGCCAT TCTCACCGBA
1021 TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT ATTTTGTACG AGGGGAAATT
1081 AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC CGATACCAGG ATCTTGCCAT
1141 CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG AAACGGCTTT TCAAAAATA
1201 TGGTATTGAT AATCCTGATA TGAATAAATG GCAGTTTCAT TTGATGCTCG ATGAGTTTTT
1261 CTAATCAGAA TTGGTTAATT GGTGTAACA CTGGCAGAGC ATTACGCTGA CTTGACGGGA
1321 CGGCNCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT
1381 AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA
1441 AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT
1501 TTTTCCGAAG GTAACGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA
1561 GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT
1621 AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGCAGC
1681 AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA
1741 GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA
1801 AAGCGCCACG CTTCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG
1861 AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT
1921 CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG
1981 CCTATGGAAA AACGCCAGCA ACGCGGCCCT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT
2041 TGCTCACATG TTCTTCCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCTAG
2101 CTGGATCGGC AAATAATGAT TTTATTTTGA CTGATAGTGA CCTGTTCTGT GCAACAAATT
2161 GATAAGCAAT GCTTTTTTAT AATGCCAACT TTGTACAAGA AAGCTGAACG AGAAACGTAA
2221 AATGATATAA ATATCAATAT ATTAAATTAG ATTTTGCATA AAAACAGAC TACATAATAC
2281 TGTAAACAC AACATATCCA GTCACATAGA TTCAACTACT TAGATGGTAT TAGTGACCTG
2341 TAGTCGACTA AGTTGGCAGC ATCACCAGAC GCACTTTGCG CCGAATAAAT ACCTGTGACG
2401 GAAGATCACT TCGCAGAATA AATAAATCCT GGTGTCCCTG TTGATACCGG GAAGCCCTGG
2461 GCCAACTTTT GCGGAAAATG AGACGTTGAT CGGCACATT CACAACCTTT ATACTTTTCT
2521 CTTACAAGTC GTTCGGCTTC ATCTGGATTT TCAGCCTCTA TACTTACTAA ACGTGATAAA
2581 GTTTCTGTAA TTTCTACTGT ATCGACCTGC AGACTGGCTG TGTATAACGG AGCCTGACAT
2641 TTATATTCCC CAGAATATCA GGTTAATGGC GTTTTGTATG TCATTTTCGC GGTGGCTGAG
2701 ATCAGCCACT TCTTCCCCGA TAACGGAGAC CGGCACACTG GCCATATCGG TGGTCATCAT
2761 GCGCCAGCTT TCATCCCCGA TATGCACCAC CGGGTAAAGT TCACGGGAGA CTTTATCTGA
2821 CAGCAGACGT GCACTGGCCA GGGGGATCAC CATCCGTCGC CCGGGCGTGT CAATAATATC
2881 ACTCTGTACA TCCACAAACA GACGATAACG GCTCTCTCTT TTATAGGTGT AAACCTTAAA
2941 CTGACATTTCA CCAGTCCCTG TTCTCGTCAG CAAAAGAGCC GTTCATTTC AATAACCGGG
3001 CGACCTCAGC CATCCCTTCC TGATTTTCCG CTTTCCAGCG TTCGGCACGC AGACGACGGG
3061 CTTCACTCTG CATGGTTGTG CTTACCAGAC CGGAGATATT GACATCATAT ATGCCTTGAG
3121 CAACTGATAG CTGTCGCTGT CAACTGTCAC TGTAATACGC TGCTTCATAG CACACCTCTT-
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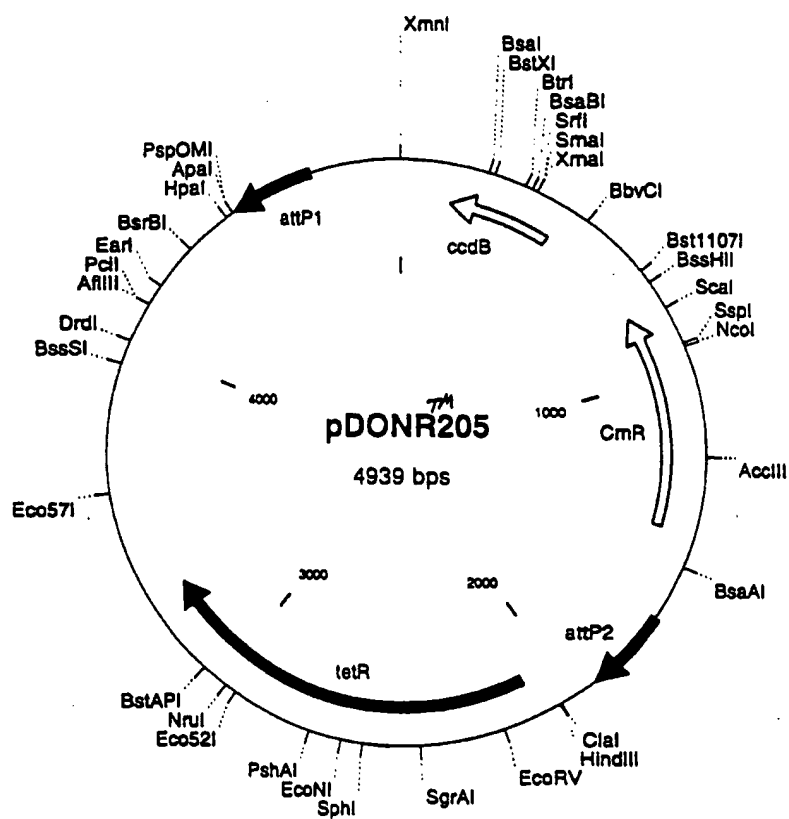
FIGURE 52B

3181 TTTGACATAC TTCGGGTATA CATATCAGTA TATATTCTTA TACCGCAAAA ATCAGCGCGC  
3241 AAATACGCAT ACTGTTATCT GGCTTTTAGT AAGCCGGATC CACGCGTTTA CGCCCCGCCC  
3301 TGCCACTCAT CGCAGTACTG TTGTAATTCA TTAAGCATTG TGCCGACATG GAAGCCATCA  
3361 CAGACGGCAT GATGAACCTG AATCGCCAGC GGCATCAGCA CCTGTGCGCC TTGCGTATAA  
3421 TATTTGCCCA TGGTGAAAAC GGGGGCGAAG AAGTTGTCCA TATTGGCCAC GTTTAAATCA  
3481 AAACCTGGTGA AACTCACCCA GGGATTGGCT GAGACGAAAA ACATATTCTC AATAAACCCCT  
3541 TTAGGGAAAT AGGCCAGGTT TTCACCGTAA CACGCCACAT CTTGCGAATA TATGTGTAGA  
3601 AACTGCCGGA AATCGTCGTG GTATTCACTC CAGAGCGATG AAAACGTTTC AGTTTGCTCA  
3661 TGGAAAACGG TGTAACAAGG GTGAACACTA TCCCATATCA CCAGCTCACC GTCTTTCATT  
3721 GCCATACGGA ATTCCGGATG AGCATTTCATC AGGCGGGCAA GAATGTGAAT AAAGGCCGGA  
3781 TAAAACTTGT GCTTATTTTT CTTTACGGTC TTTAAAAGG CCGTAATATC CAGCTGAACG  
3841 GTCTGGTTAT AGGTACATTG AGCAACTGAC TGAAATGCCT CAAAATGTTT TTTACGATGC  
3901 CATTGGGATA TATCAACGGT GGTATATCCA GTGATTTTTT TCTCCATTTT AGCTTCCTTA  
3961 GCTCCTGAAA ATCTCGATAA CTCAAAAAAT ACGCCCGGTA GTGATCTTAT TTCATTATGG  
4021 TGAAAGTTGG AACCTCTTAC TGTTCTTGAT GCAGATGATT TTCAGGACTA TGACACTAGC  
4081 ATATATGAAT AGGTAGATGT TTTTATTTTG TCACACAAAA AAGAGGCTCG CACCTCTTTT  
4141 TCTTATTTCT TTTTATGATT TAATA

FIGURE 52C

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Figure 53A: pDONR205 (tetR)





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## pDONR205 4939 bp

GGCATCAGCACCTTGTGCGCTTGCCTATAATATTTGCCCATGGTGAAAACGGGGGCGAAG  
AAGTTGTCCATATTGGCCACGTTTTAAATCAAACCTGGTGAACTCACCCAGGGATTGGCT  
GAGACGAAAAACATATTCTCAATAAACCCCTTTAGGGAAATAGGCCAGGTTTCACCGTAA  
CACGCCACATCTTGCGAATATATGTGTAGAACTGCCGGAAATCGTCGTGGTATTCATC  
CAGAGCGATGAAAACGTTTCAGTTTGCTCATGAAAACGGTGTAACAAGGGTGAACACTA  
TCCCATATCACCAGCTCACCGTCTTTTCATTGCCATACGGAATTCGGGATGAGCATTATC  
AGGCGGGCAAGAATGTGAATAAAGCCGGATAAACTTGTGCTTATTTTTCTTTACGGTC  
TTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGAC  
TGAAATGCCCTCAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCA  
GTGATTTTTTTCTCCATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAAT  
ACGCCCGGTAGTGATCTTATTTTCATTATGGTGAAAGTTGGAACTCTTACGTGCCGATCA  
ACGTCTCATTTTTGCCCAAAAGTTGGCCACAGGGCTTCCCGGTATCAACAGGGACACCAGGA  
TTTATTTATTCTGCGAAGTGATCTTCCGTACAGGTATTTATTTCGGCGCAAGTGCGTCG  
GGTGATGCTGCCAACTTAGTCGACTACAGGTCACTAATACCATCTAAGTAGTTGATTAT  
AGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTATGCAAAATCTAA  
TTAATATATTGATATTATATCATTTTACGTTTCTCGTTCAGCTTTCTTGACAAAGTT  
GGCATTATAAGAAAGCATTGCTTATCAATTTGTTGCAACGAACAGGTCACTATCAGTCAA  
AATAAAATCATTATTTGCCATCCAGCTGCAGCTCTGGCCCGTGTCTCAAAATCTCTGATG  
TTACATTGCACAAGATAAAAAATATATCATCATGAATTTCTCATGTTTGACAGCTTATCATC  
GATAAGCTTTAATGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAGGCACCGTGT  
ATGAAATCTAACAATGCGCTCATCGTCATCCTCGGCACCGTCACCCCTGGATGCTGTAGGC  
ATAGGCTTGGTTATGCCGGTACTGCCGGGCTCTTGCGGGATATCGTCCATTCGGACAGC  
ATCGCCAGTCAGTATGGCGTGCTGTAGCGCTATATGCGTTGATGCAATTTCTATGCCGA  
CCCGTTCTCGGAGCACTGTCCGACCGCTTTGGCCGCCGCCAGTCCTGCTCGCTTCGCTA  
CTTGAGGCCACTATCGACTACGCGATCATGGCGACCACACCCGTCCTGTGGATCCTCTAC  
GCCGACGATCGTGGCCGGCATCCCGCGCCACAGGTGCGGTGCTGCGCGCTATATC  
GCCGACATACCCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTC  
GGCGTGGGTATGGTGGCAGGCCCGGTGGCCGGGGGACTGTTGGGCGCCATCTCCTTGCA  
GCACCATTCCTTGCGGCGCGGTGCTCAACGGCTCAACCTACTACTGGGCTGCTTCCTA  
ATGCAGGAGTCGCATAAGGGAGAGCGTCGACCGATGCCCTTGAGAGCCTTCAACCCAGTC  
AGCTCCTTCGGGTGGGCGCGGGGATGACTATCGTCGCCGCACTTATGACTGTCTTCTTT  
ATCATGCAACTCGTAGGACAGGTGCCGGCAGCGCTCTGGGTCATTTTCGGCGAGGACCGC  
TTTCGCTGGAGCGCGACGATGATCGGCCTGTGCTTGGCGTATTCGGAATCTTGACGCC  
CTCGCTCAAGCTTTCGTCAGTGGTCCCGCCACCAACGTTTCGGCGAGAAGCAGGCCATT  
ATCGCCGGCATGGCGGCCGACGCGCTGGGCTACGTCTTGCTGGCGTTTCGCGACGCGAGGC  
TGGATGGCCTTCCCCATTATGATTCTTCTCGCTTCGGCGGCATCGGGATGCCCGCGTTG  
CAGGCCATGCTGTCCAGGCAGGTAGATGACGACCATCAGGGACAGCTTCAAGGATCGCTC  
GCGGCTCTTACCAGCCTAACTTCGATCATTGGACCGCTGATCGTCACGGCGATTTATGCC  
GCCTCGGCGAGCATGGAACGGGTGGCATGGATTGTAGGCGCCGCCCTATACCTTGTC  
TGCCCTCCCCGCGTTGCGTCGCGGTGCATGGAGCCGGGCCACCTCGACCTGAATGGAAGCC  
GGCGGCACCTCGCTAACGGATTCAACACTCCAAGAATTGGAGCCAATCAATTCTTGCGGA  
GAACTGTGAATGCGCAAAACCAACCCTTGGCAGAACATATCCATCGCATGACCAAAATCCC  
TTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAGGATCTTC  
TTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACCCCGCTACC  
AGCGGTGTTTTGTTTGGCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTT  
CAGCAGAGCGCAGATACCAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTT  
CAAGAATCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAAGTGGCTGC  
TGCCAGTGGCGATAAGTCGTGCTTACCAGGTTGGACTCAAGACGATAGTTACCGGATAA  
GGCGCAGCGGTGGGCTGAACGGGGGTTGCTGCACACAGCCAGCTTGGAGCGAAGCAGC  
CTACACCGAAGTGAATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGG  
GAGAAAGGCGGACAGGTATCCGGAAGCGGCAGGGTGGAAACAGGAGAGCGCAGAGGGA  
GCTTCCAGGGGGAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACT  
TGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGGAACCGCCAGCAA-

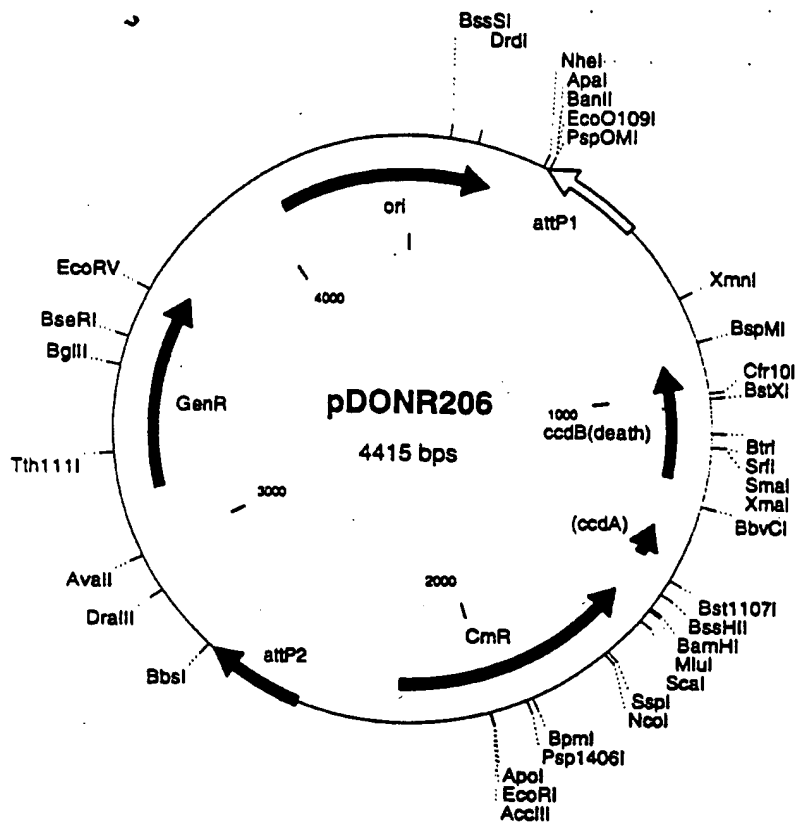
FIGURE 53B

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CGCGGCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCTG  
GTTATCCCCTGATTCTGTGGATAACCGTATTACCGCTAGCCAGGAAGAGTTTGTAGAAAC  
GCAAAAAGGCCATCCGTGAGGATGGCCTTCTGCTTAGTTTGATGCCTGGCAGTTTATGGC  
GGGCGTCCTGCCCCGCCACCTCCGGGCCGTTGCTTCACAACGTTCAAATCCGCTCCCGGC  
GGATTGTCTACTCAGGAGAGCGTTACCCGACAAACAACAGATAAAACGAAAGGCCAG  
TCTTCCGACTGAGCCTTTCGTTTTATTGTGATGCCTGGCAGTTCCCTACTCTCGCGTTAAC  
GCTAGCATGGATCTCGGGCCCCAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCG  
TTGCAACAAATTGATGAGCAATGCTTTTTTATAATGCCAACTTTGTACAAAAAGCTGAA  
CGAGAAACGTAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAACAG  
ACTACATAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATGGT  
ATTAGTGACCTGTAGTCGACCGACAGCCTTCCAAATGTTCTTCGGGTGATGCTGCCAACT  
TAGTCGACCGACAGCCTTCCAAATGTTCTTCTCAAACGGAATCGTCGTATCCAGCCTACT  
CGCTATTGTCTCAATGCCGTATTAAATCATAAAAAGAAATAAGAAAAAGAGGTGCGAGC  
CTCTTTTTTGTGTGACAAAATAAAAACATCTACCTATTTCATATACGCTAGTGTCATAGTC  
CTGAAAATCATCTGCATCAAGAACAATTTCACAACTCTTATACTTTTCTTTACAAGTCG  
TTCGGCTTCATCTGGATTTTCAGCCTCTATACTTACTAAACGTGATAAAGTTTCTGTAAT  
TTCTACTGTATCGACCTGCAGACTGGCTGTGTATAAGGGAGCCTGACATTTATATTTCCC  
AGAACATCAGGTTAATGGCGTTTTTGATGTCATTTTTCGCGGTGGCTGAGATCAGCCACTT  
CTTCCCCGATAACGGAGACCGGCACACTGGCCATATCGGTGGTCATCATGCGCCAGCTTT  
CATCCCCGATATGCACCACCGGGTAAAGTTCACGGGAGACTTTATCTGACAGCAGACGTG  
CACTGGCCAGGGGGATCACCATCCGTGCGCCGGGCGTGTCAATAATATCACTCTGTACAT  
CCACAAACAGACGATAACGGCTCTCTCTTTTATAGGTGTAAACCTTAAACTGCATTTAC  
CAGTCCCTGTTCTCGTCAGCAAAAGAGCCGTTCAATTTCAATAAACCGGGCGACCTCAGCC  
ATCCCTTCTGATTTTCCGCTTTCCAGCGTTCGGCACGCAGACGACGGGCTTCATTCTGC  
ATGGTTGTGCTTACCAGACCGGAGATATTGACATCATATATGCCTTGAGCAACTGATAGC  
TGTGCTGTCAACTGTCACTGTAATACGCTGCTTCATAGCACACCTCTTTTGACATACT  
TCGGGTATACATATCAGTATATATTCTTATACCGCAAAATCAGCGCGCAAAATACGCATA  
CTGTTATCTGGCTTTTAGTAAGCCGGATCCACGCGATTACGCCCCGCCCTGCCACTCATC  
GCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAGACGGCATG  
ATGAACCTGAATCGCCAGC

FIGURE 53C

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## pDONR206 4415 bp

CGGCATTGAGGACAATAGCGAGTAGGCTGGATACGACGATTCCGTTTGAGAAGAACATTT  
GGAAGGCTGTCGGTCGACTACAGGTCATAATACCATCTAAGTAGTTGAATCATAGTGAC  
TGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTATGCAAAATCTAATTTAAT  
ATATTGATATTTATATCATTTTACGTTTCTCGTTTCTCGTTTGTACAAAGTTGGCATT  
ATAAAAAAGCATTGCTTATCAATTTGTTGCAACGACAGGTCACTATCAGTCAAAATAAA  
ATCATTATTTGGGGCCCCGAGATCCATGCTAGCGGTAATACGGTTATCCACAGAATCAGGG  
GATAACGCGAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAG  
GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGA  
CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCT  
GGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCGGCTTACCGGATACCTGTCCGCC  
TTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGGCTGTAGGTATCTCAGTTTCG  
GTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTTACGCCCCGACCGC  
TGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA  
CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAG  
TTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCT  
CTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACC  
ACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGA  
TCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCA  
CGTTAAGGGATTTTGGTTCATGNCGCCGTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGT  
TACAACCAATTAAACCAATTCTGATTAGAAAACTCATCGAGCATCAAATGAACTGCAAT  
TTATTATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGA  
GAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCG  
ACTCGTCCAACATCAATACAACCTATTAGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGC  
AGATCCGTGCACAGCACCTTGCCGTAGAAGAACAGCAAGGCCGCCAATGCCTGACGATGC  
GTGGAGACCGAAACCTTGCGCTCGTTCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTG  
CTGCCCAAGGTTGCGGGTGACGCACACCGTGGAACGGATGAAGGCACGAACCCAGTTG  
ACATAAGCCCTGTTCCGGTTCGTAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGG  
TCCAGAACCTTGACCGAACGCGAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGT  
TATGACTGTTTTTTGTACAGTCTATGCCCTCGGGCATCCAAGCAGCAAGCGGTTACGCC  
GTGGGTGATGTTTGATGTTATGGAGCAGCAACGATGTACGCAGCAGCAACGATGTTAC  
GCAGCAGGGCAGTCCGCCCTAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTGCGAC  
ATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCTG  
TGAGTTCGGAGACGTAGCCACCTACTCCCAACATCAGCCGGAATCCGATTACCTCGGGAA  
CTTGCTCCGTAGTAAGACATTATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGG  
CGCTCTCGCGGCTTACGTTCTGCCAGGTTTGAGCAGCCGCTAGTGAGATCTATATCTA  
TGATCTCGCAGTCTCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCT  
CCTCAAGCATGAGGCCAACGCGCTTGGTGCTTATGTGATCTACGTGCAAGCAGATTACGG  
TGACGATCCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTT  
TGATATCGACCCAAGTACCGCCACCTAACCAATTCGTTCAAGCCGAGATCGGCTTCCCGGC  
CTAATTTCCCTCGTCAAAAAAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTG  
AATCCGGTGAGAAATGGCAAAAGCGTATGCATTTCTTTCCAGACTTGTTCACAGGCCAGC  
CATTACGCTCGTCATCAAAATCACTCGCATCAACCAACCGTTATTCAATTCGTGATTGCG  
CCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAAT  
GCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATT  
CTTCTAATACCTGGAATGCTGTTTTCCCGCGGATCGCAGTGGTGAGTAACCATGCATCAT  
CAGGAGTACGGATAAAATGCTTGATGGTCCGAAGAGGCATAAATCCGTGAGCCAGTTTA  
GTCTGACCATCTCATCTGTAACATCATTTGCAACGCTACCTTTGCCATGTTTCAGAAACA  
ACTCTGGCGCATCGGGCTTCCCATACAATCGAAAGATTGTGCGACCTGATTGCCCGACAT  
TATCCGAGGCCCATTTATACCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCC  
TCCAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGT  
AAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTGTGCAATGTAACATCAGA  
GATTTTGAGACACGGGCCNCGCGCACTGCAGCTGGATCGGCAATAATGATTTTATTTTG  
ACTGATAGTGACCTGTTGTTGCAACAAATTGATAAGCAATGCTTTTTTATAATGCCAAC -

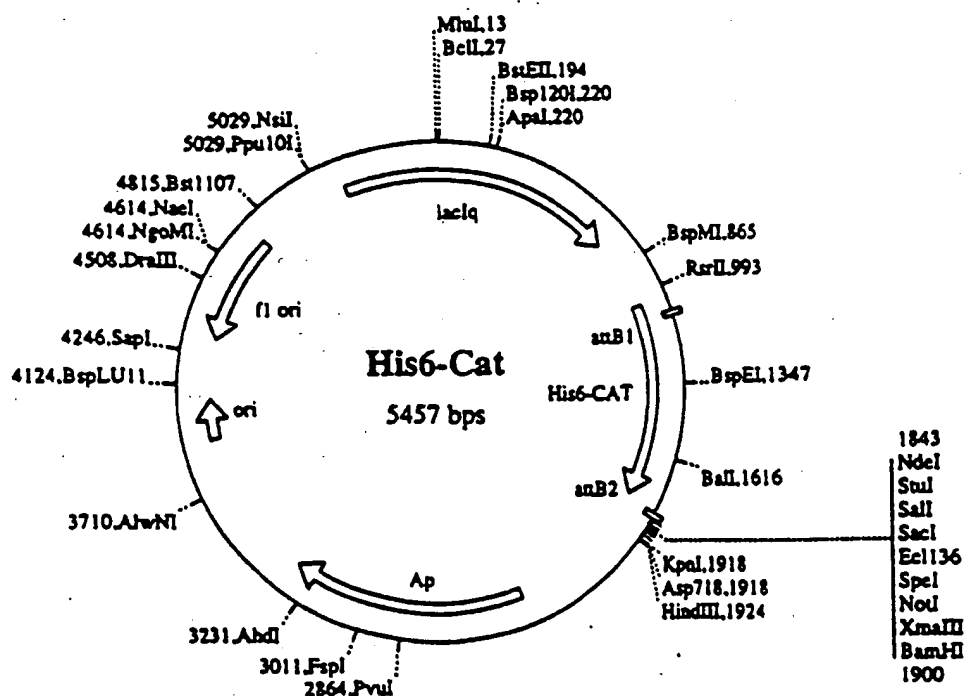
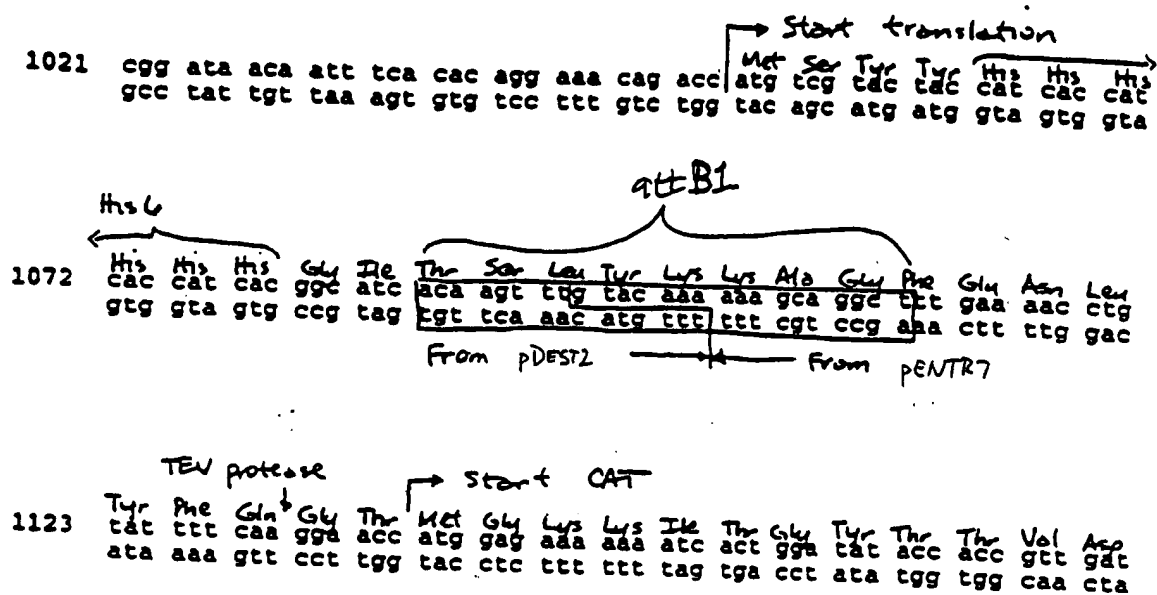
FIGURE 54B

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TTTGTACAAGAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTA  
GATTTTGCATAAAAAACAGACTACATAAATACTGTAAAACACAACATATCCAGTCACTATG  
ATTCAACTACTTAGATGGTATTAGTGACCTGTAGTCGACTAAGTTGGCAGCATCACCCGA  
CGCACTTTGCGCCGAATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCC  
TGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGA  
TCGGCAGCTAAGAGGTTCCAACCTTTCACCATAATGAAATAAGATCACTACCGGGCGTATT  
TTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGG  
ATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTCAGTC  
AGTTGCTCAATGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGAC  
CGTAAAGAAAAATAAGCACAAAGTTTTATCCGGCCTTTATTACATTCTTGCCCGCCTGAT  
GAATGCTCATCCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAG  
TGTTCAACCCTTGTTACACCGTTTTCCATGAGCAAACGTAAACGTTTTTCATCGCTCTGGAG  
TGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCCGAAGATGTGGCGTGTTA  
CGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGC  
CAATCCCTGGGTGAGTTTTCACCACTTTTGATTTAAACGTGGCCAAATATGGACAACTTCTT  
CGCCCCCGTTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCT  
GGCGATTCAAGGTTTCATCATGCCGCTCTGTGATGGCTTCCATGTCCGCAGAATGCTTAATGA  
ATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAACGCGTGATCCGGCTTACT  
AAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATAC  
TGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTG  
ACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTC  
TGTAAGCACAAACCATGCAGATGAAGCCCGTCGTCTGCGTGCCGAACGCTGGAAAGCGG  
AAAATCAGGAAGGGATGGCTGAGGTGCGCCGGTTTTATTGAAATGAACGGCTCTTTTGCTG  
ACGAGAACAGGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGT  
TATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACGGATGGTG  
ATCCCCCTGGCCAGTGACGCTGTGCTGTGAGATAAAGTCTCCCGTGAACCTTACCCGGTG  
GTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTCGGGTC  
TCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCC  
ATTAACCTGATGTTCTGGGGAATATAAATGTCAAGGCTCCGTTATACACAGCCAGTCTGCA  
GGTCGATACAGTAGAAAATTACAGAACTTTATCACGTTTAGTAAGTATAGAGGCTGAAAA  
TCCAGATGAAGCCGAACGACTTGTAAGAGAAAAGTATAAGAGTTGTGAAATTGTTCTTGA  
TGCAGATGATTTTCAGGACTATGACACTAGCATATATGAATAGGTAGATGTTTTATTTT  
GTCACACAAAAAGAGGCTCGCACCTCTTTTCTTATTTCTTTTATGATTTAATA

FIGURE 54 C

Figure 55 An Entry (pENTR7) Clone of CAT Subcloned into pDEST2



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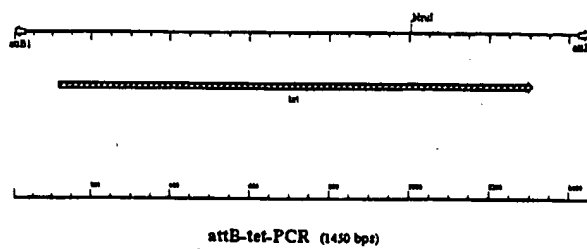
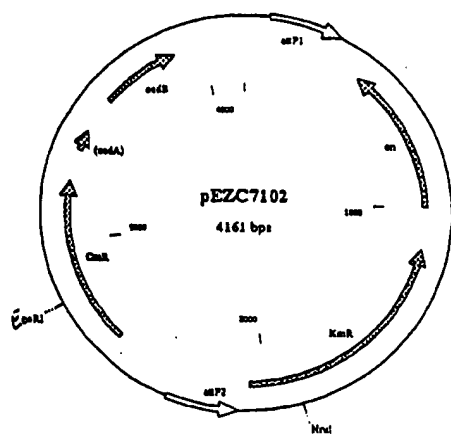


FIGURE 56

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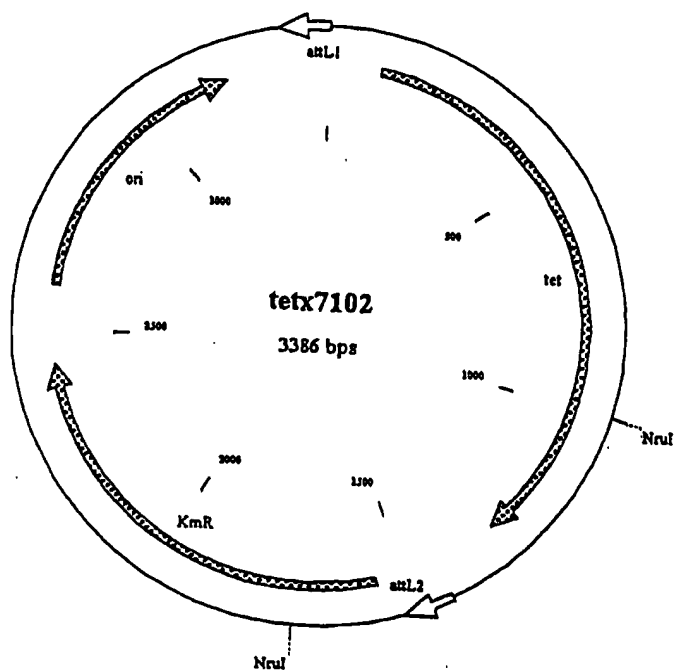


FIGURE 57





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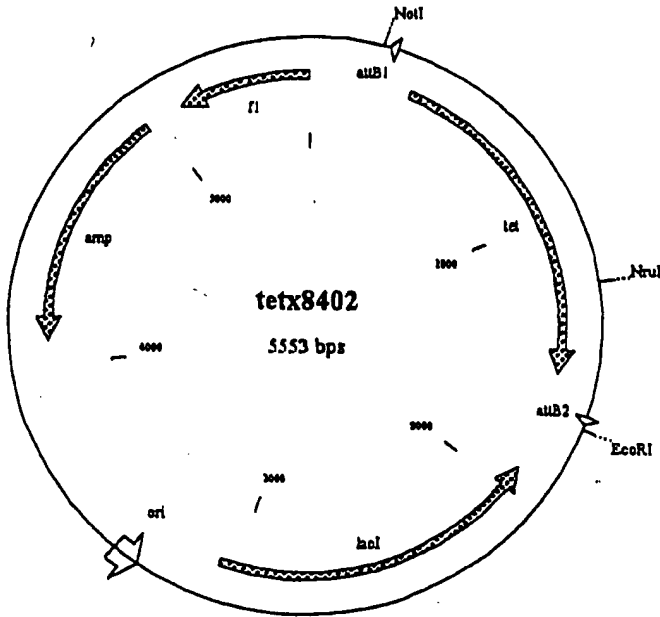


FIGURE 59

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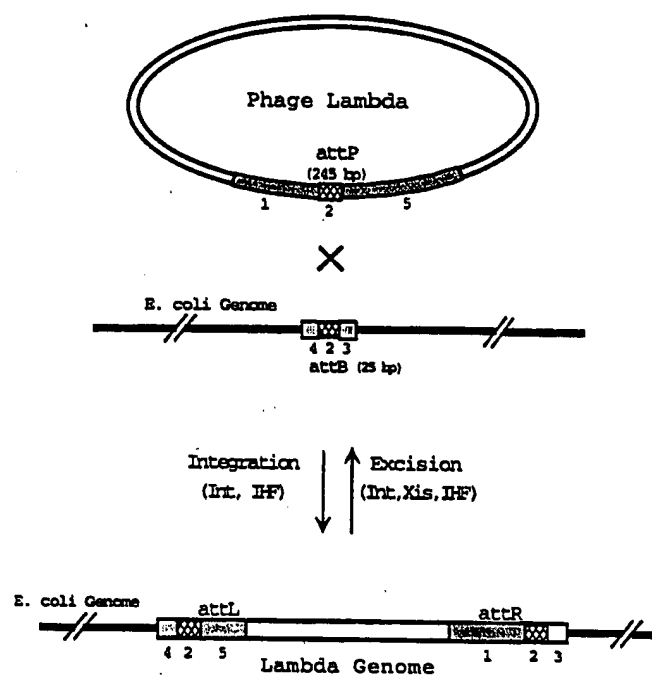


FIGURE 60

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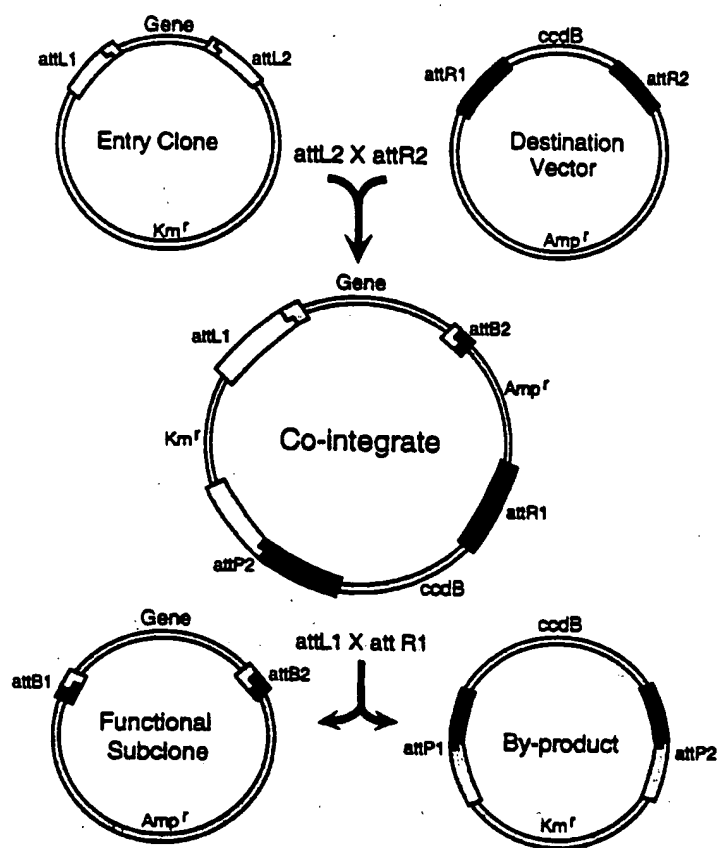
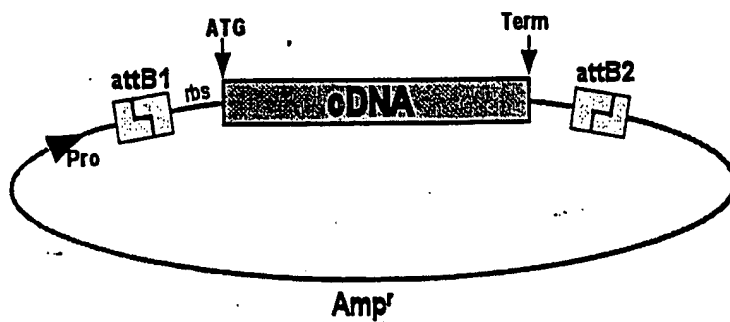


FIGURE 61

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### Native Protein Expression:



### Fusion Protein Expression:

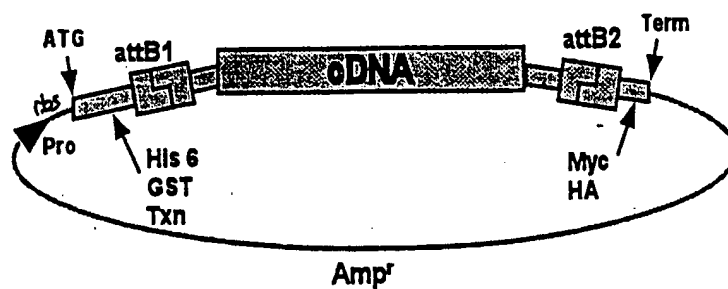


FIGURE 62

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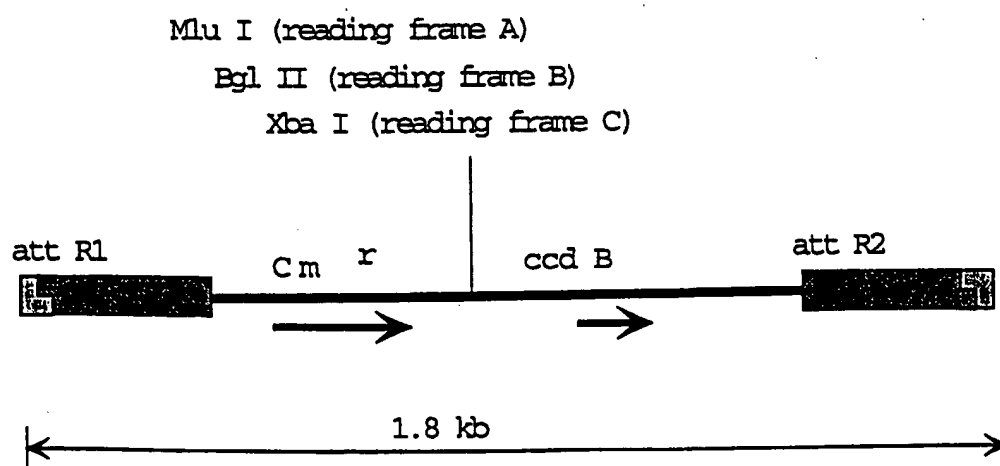
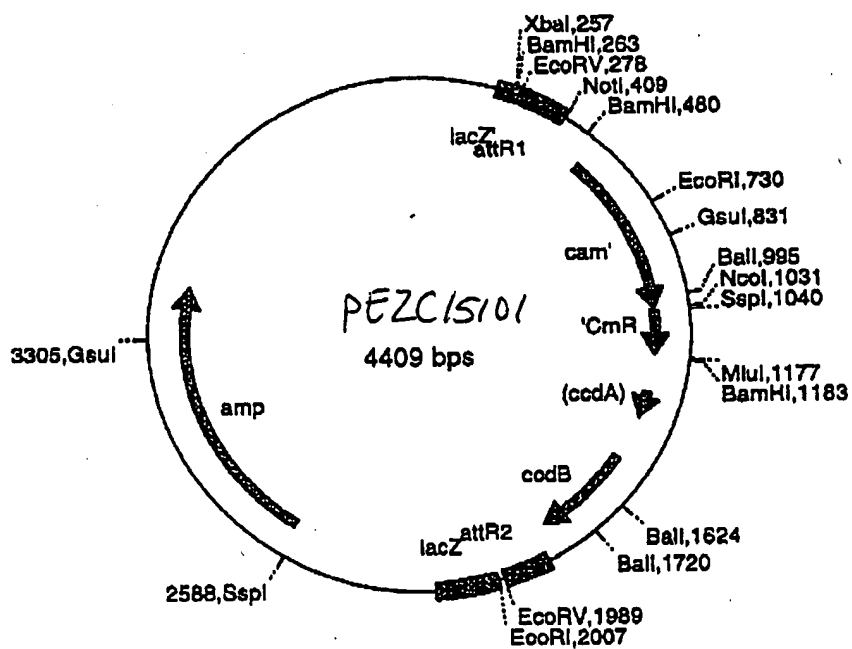


FIGURE 63

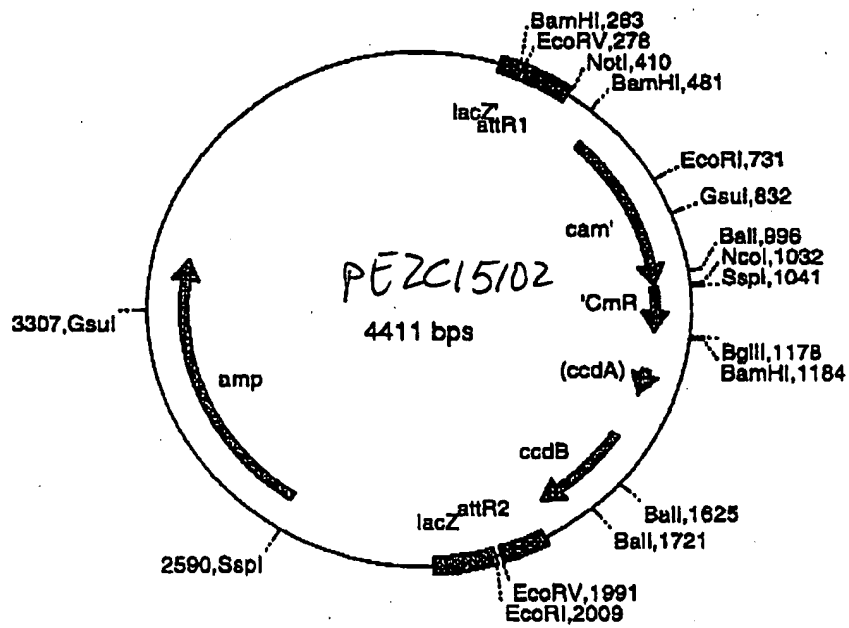
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FIGURE 64A



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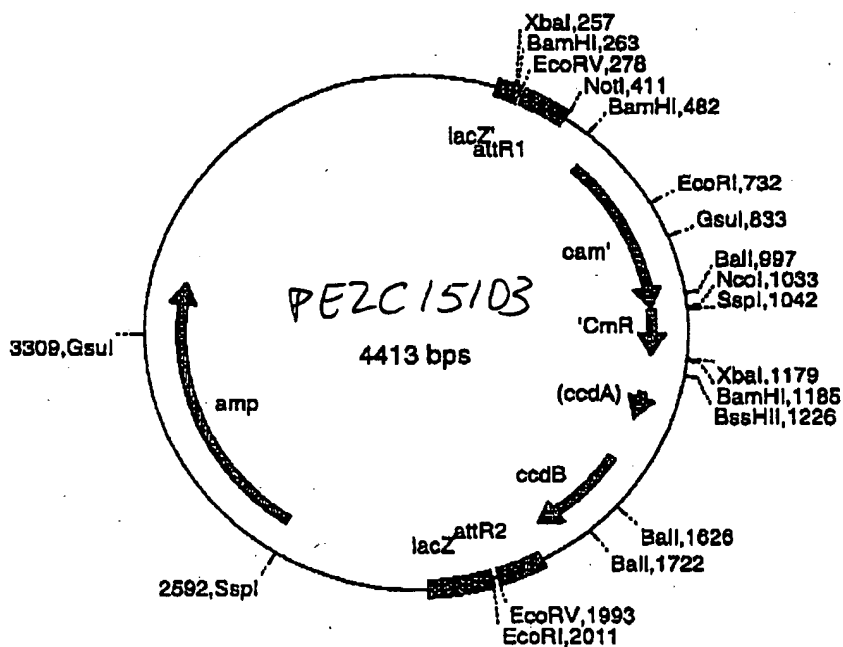
FIGURE 4A





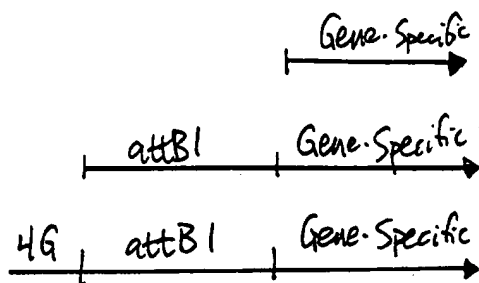
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FIGURE 64C



# Primers for Amplifying *tetR* and *ampR* for Cloning by Recombination

## Primers



## Reverse Primers

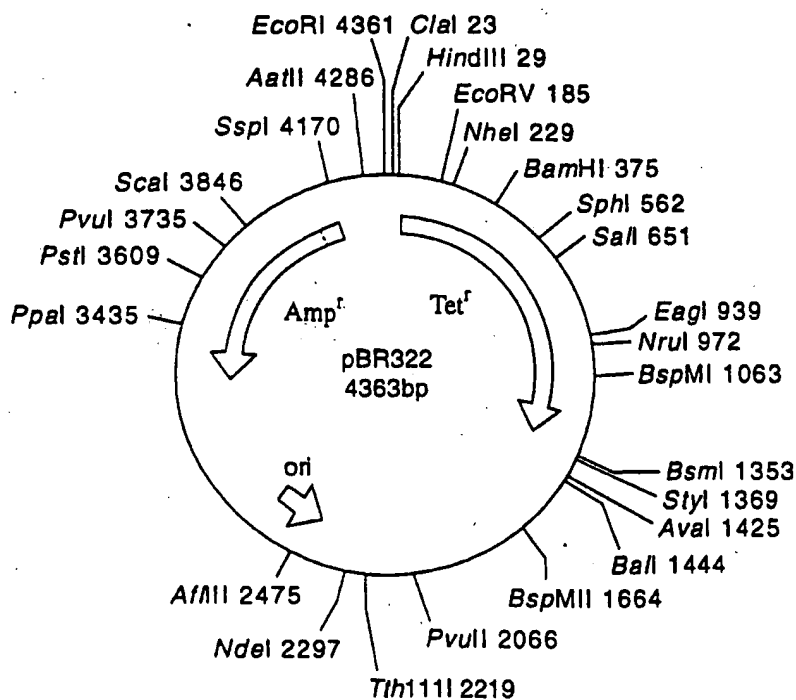
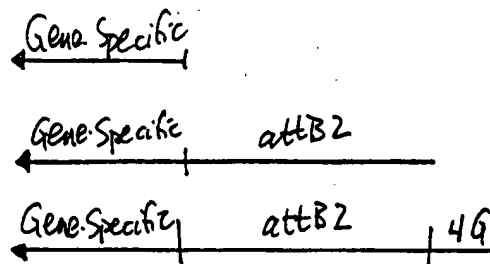


FIGURE 65

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**Results of Cloning  
tet and amp PCR Products  
by Recombination**

PCR Product Used in GCS Reactions	No. Colonies Obtained (100 ul plated)	Form of DNA Analyzed	Colonies Obtained of Predicted Size
tet	6, 10	SC	0 of 8
attB-tet	9, 6	SC	1 of 8
attB+4G-tet	824, 1064	SC AvaI+Bam	7 of 7 7 of 7
amp	7, 13	SC	0 of 8
attB-amp	18, 22	SC	3 of 8
attB+4G-amp	3020, 3540	SC PstI	8 of 8 8 of 8
attB Plasmid (Pos. Control)	320, 394		

FIGURE 66

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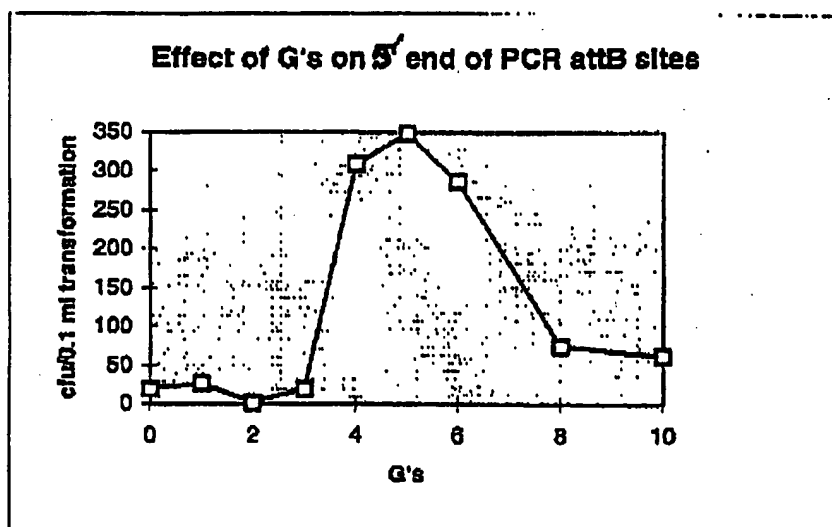


FIGURE 67

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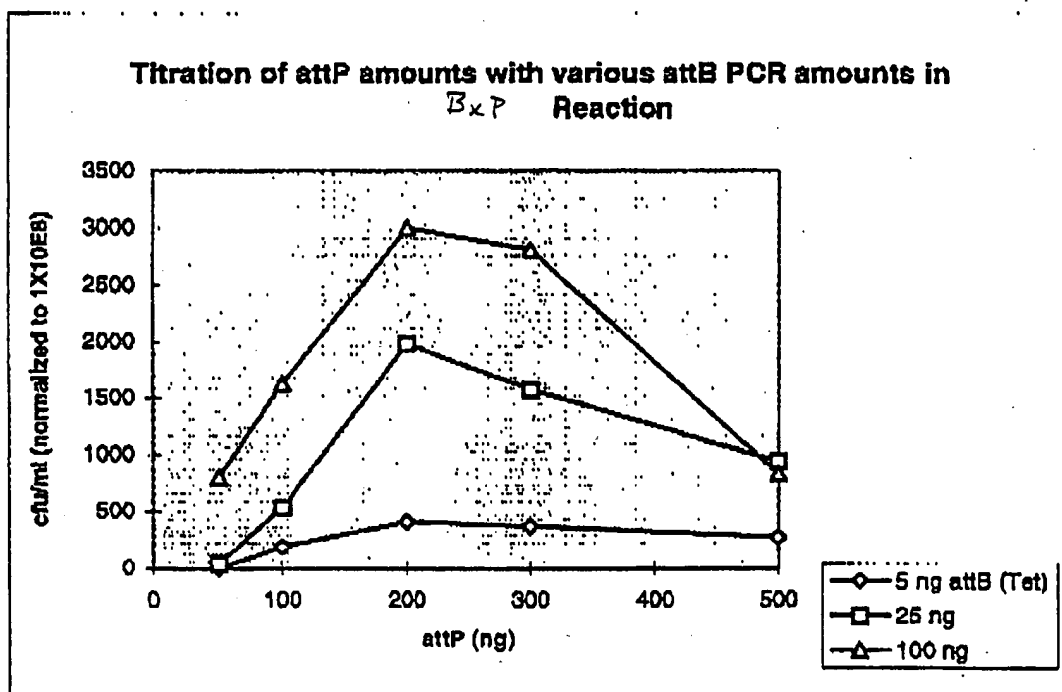
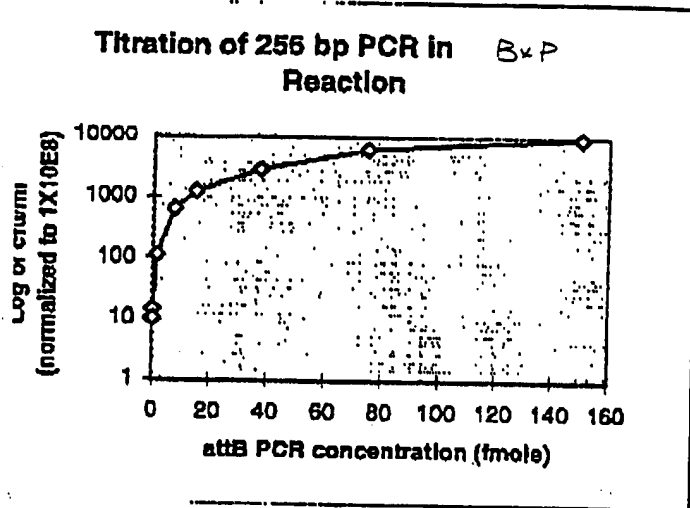


FIGURE 68

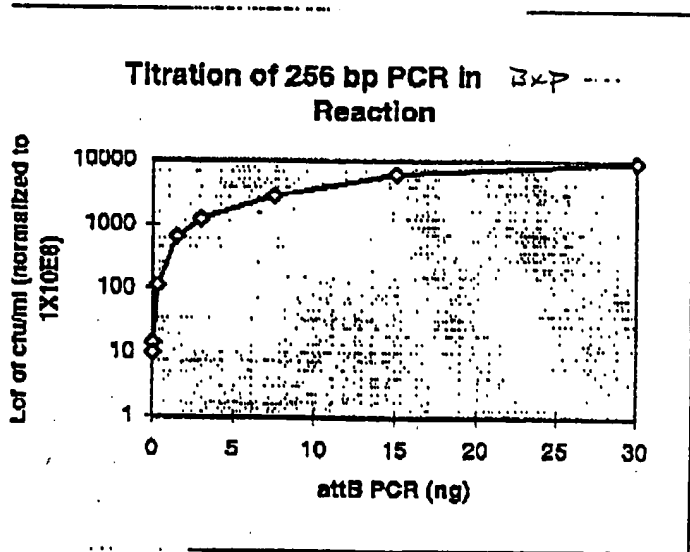
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FIGURE  
69

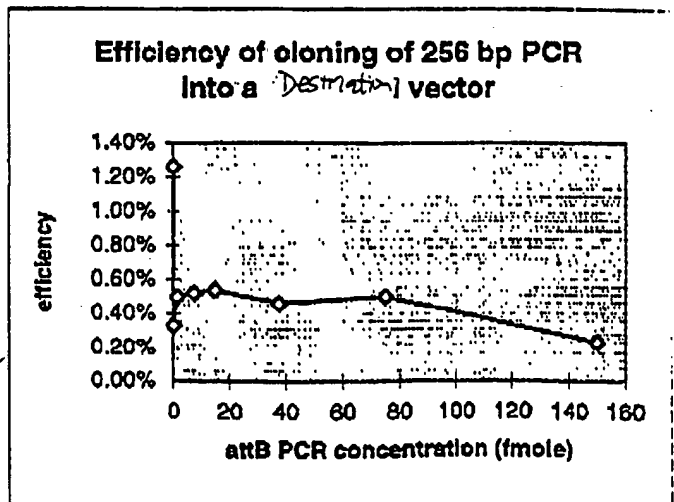
A



B



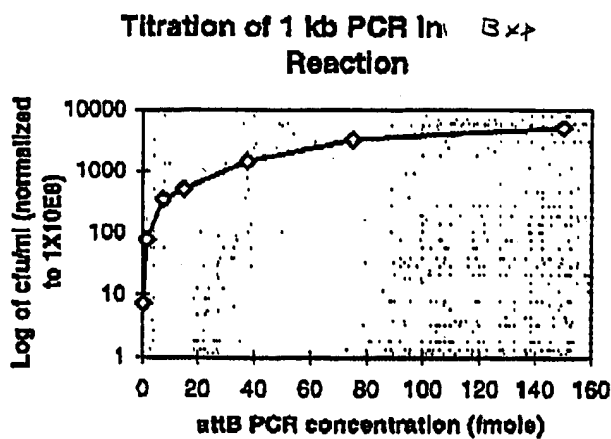
C



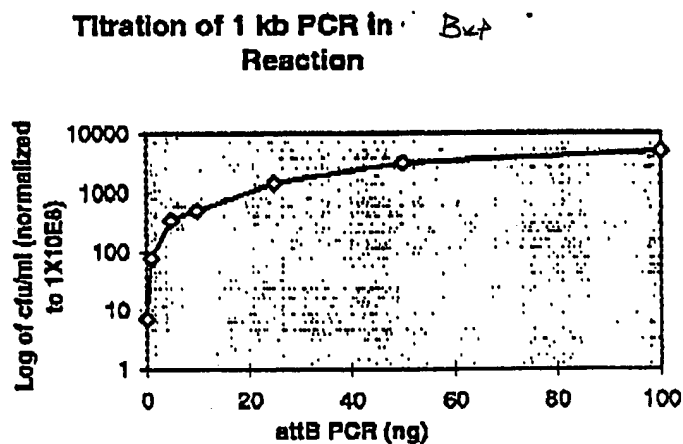
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FIGURE  
70

A



B



C

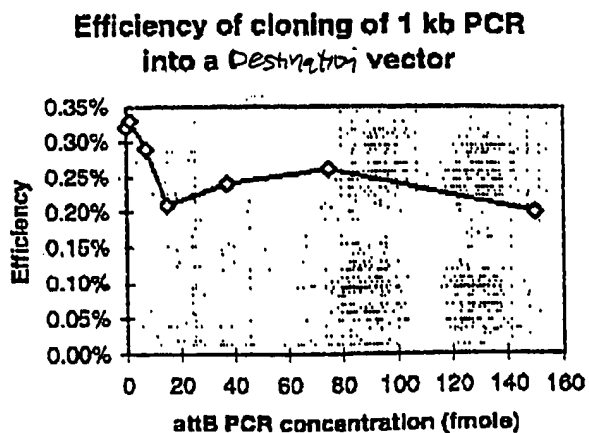
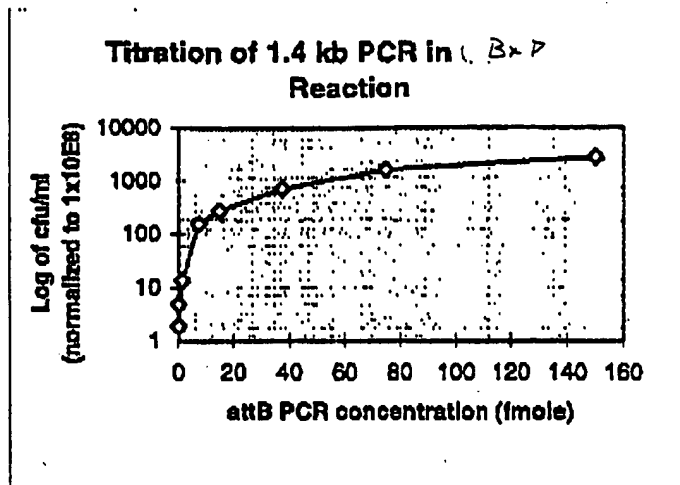
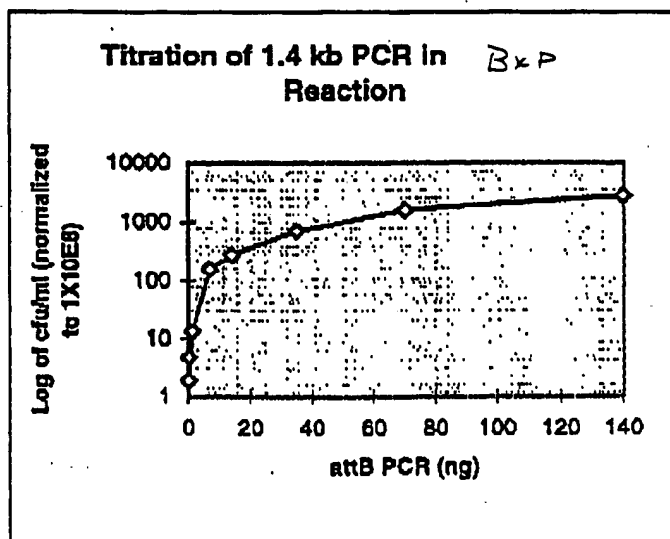


FIGURE 71

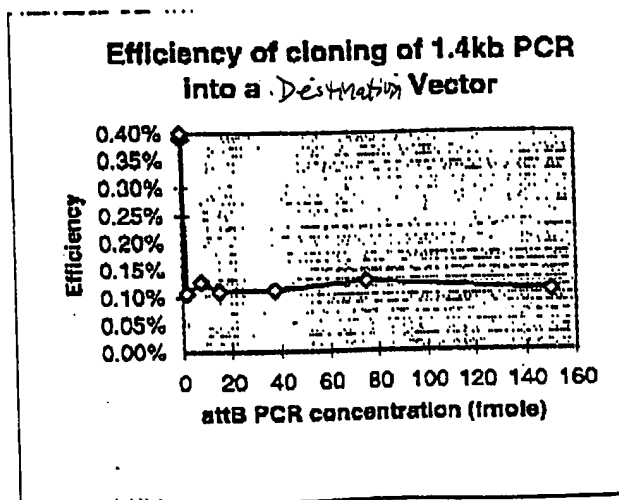
A



B



C

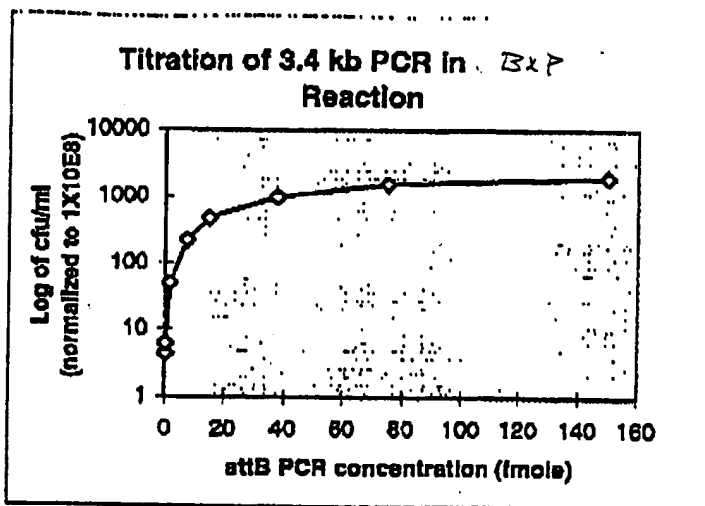




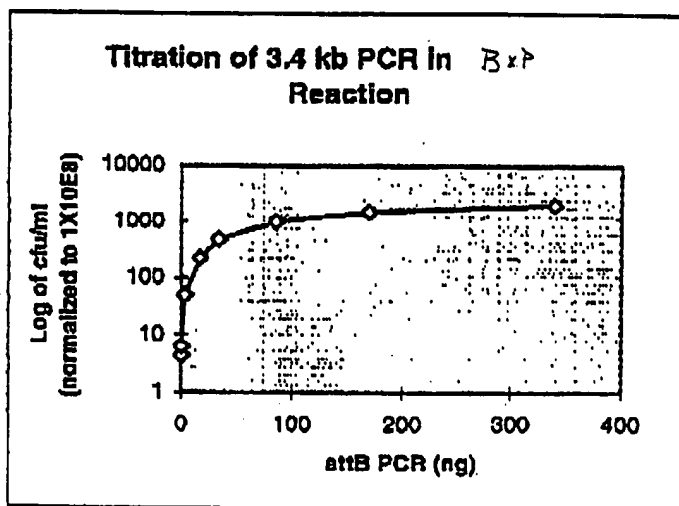
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FIGURE 72

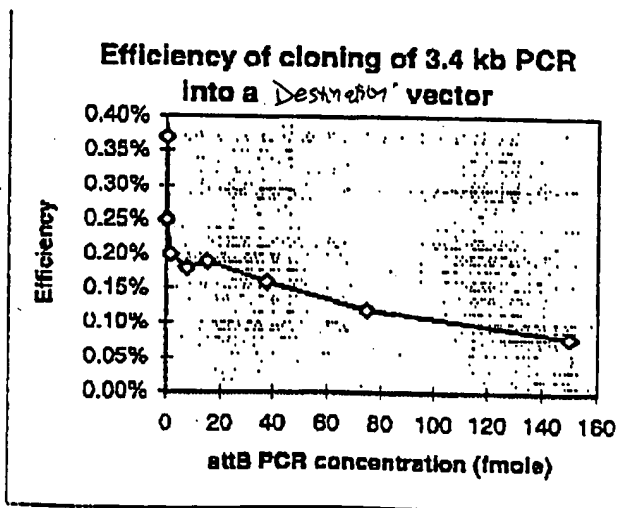
A



B



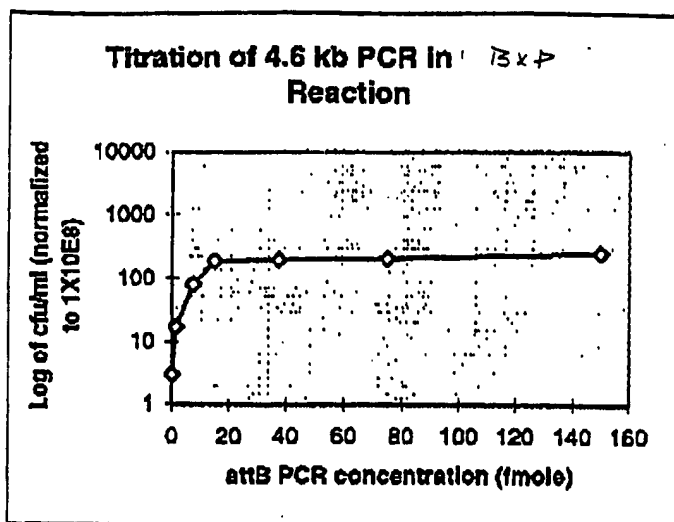
C



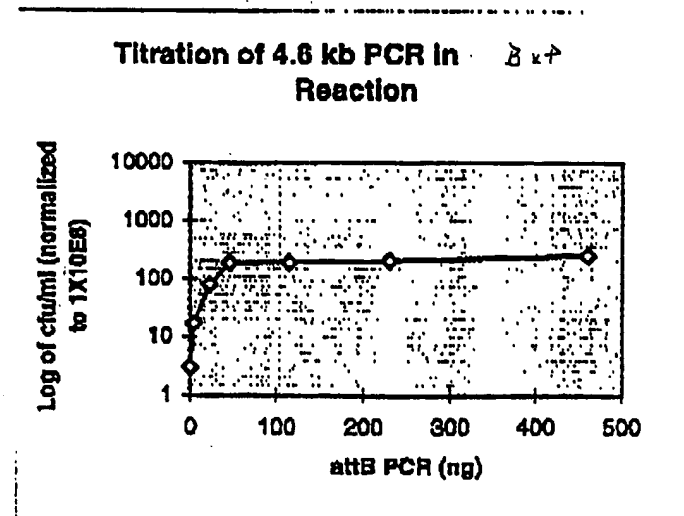
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FIGURE 73

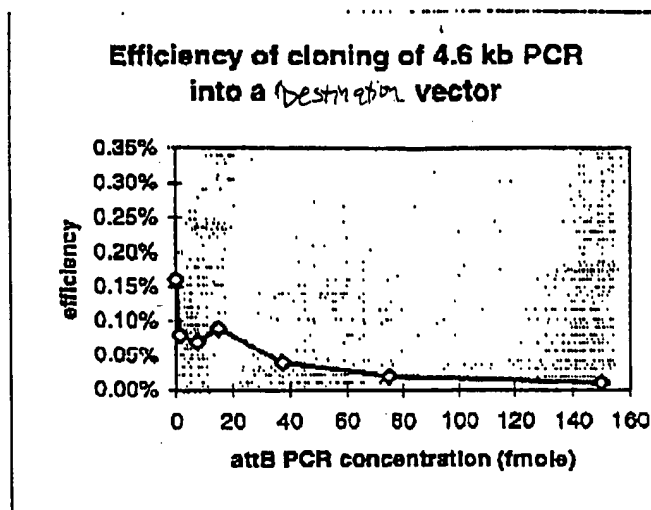
A



B



C



6.9 kb PCR DNA Titration in  $\alpha$  BxP Reaction

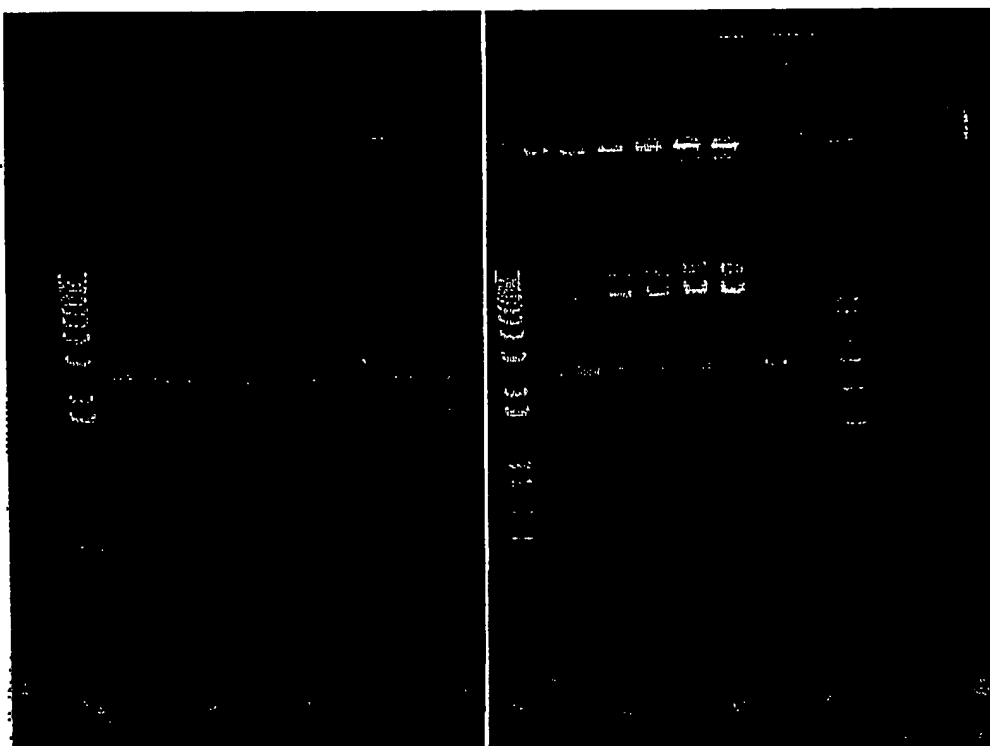


FIGURE 74

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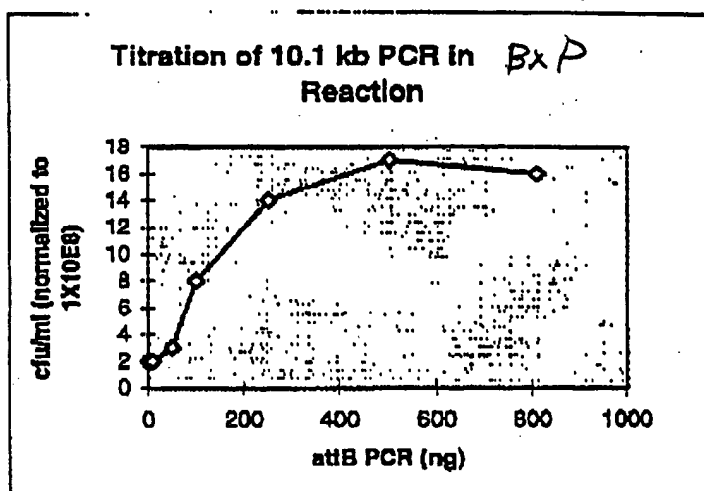
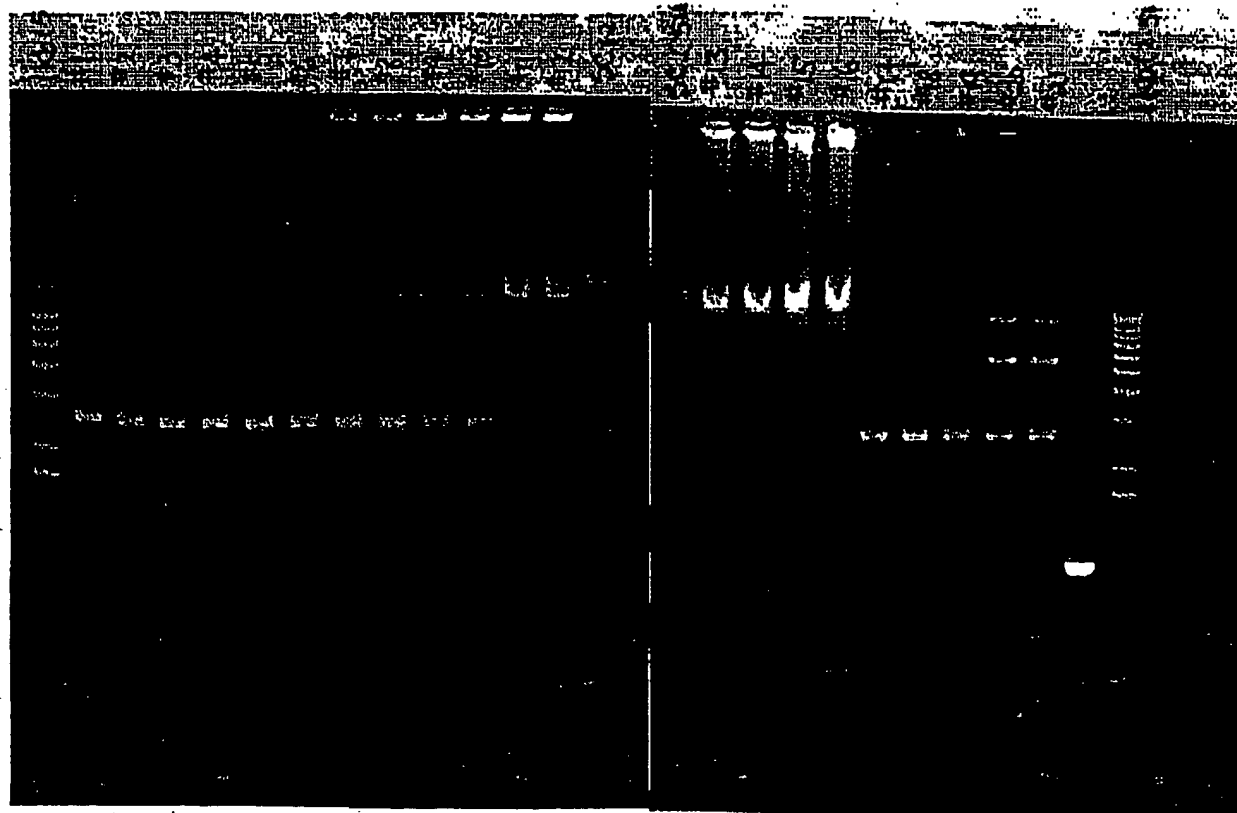


FIGURE 75-

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# 10.1 kb PCR DNA Titration in BxP Reaction



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FIGURE 76

### Cloning of PCR Products of Different Sizes with the GATEWAY™ PCR Cloning System

Size	fmols PCR DNA	ng PCR DNA	Cols/ml Transformation (pUC=10 <sup>8</sup> CFU/ml)	Correct Clones/Total Examined**
0.26 kb*	15	3	1223	10/10 (a)
	37.5	7.5	2815	
1.0 kb	15	10	507	49/50 (b)
	37.5	25	1447	
1.4 kb	15	14	271	48/50 (c)
	37.5	35	683	
3.4 kb	15	34	478	9/10 (a)
	37.5	85	976	
4.6 kb	15	46	190	10/10 (a)
	37.5	115	195	
6.9 kb	15	69	30 (235)**	47/50 (b)
	37.5	173	54 (463)**	

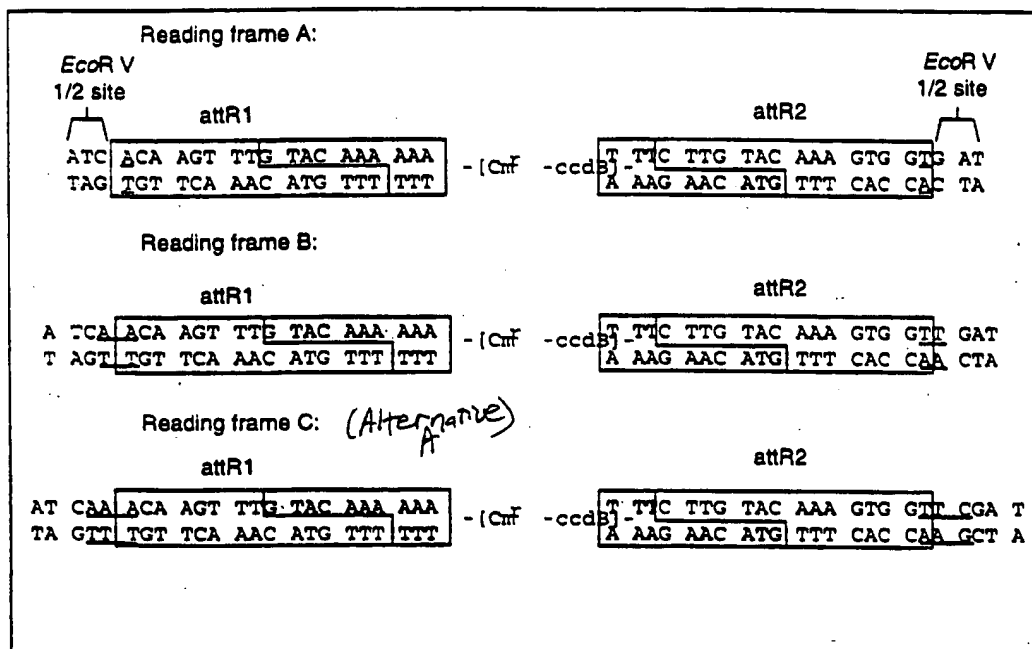
\*The 0.26 kb PCR product was used unpurified; all the others were purified by precipitation with PEG/MgCl<sub>2</sub> as described in the text of Example 9, to remove primer dimers potentially present. Standard incubations were for 60 min.

\*\*overnight incubation

- (a) DNA minipreps
- (b) ampR/kanR
- (c) tetR/kanR

**Figure 77**

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Reading frame C: (Alternative)

<sub>B</sub>

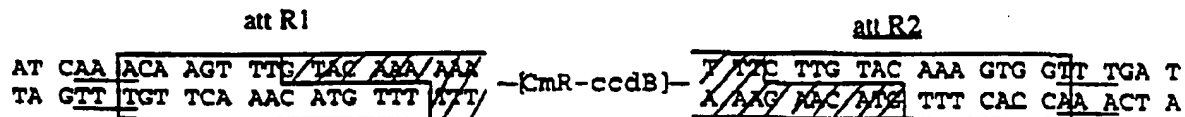


FIGURE 78

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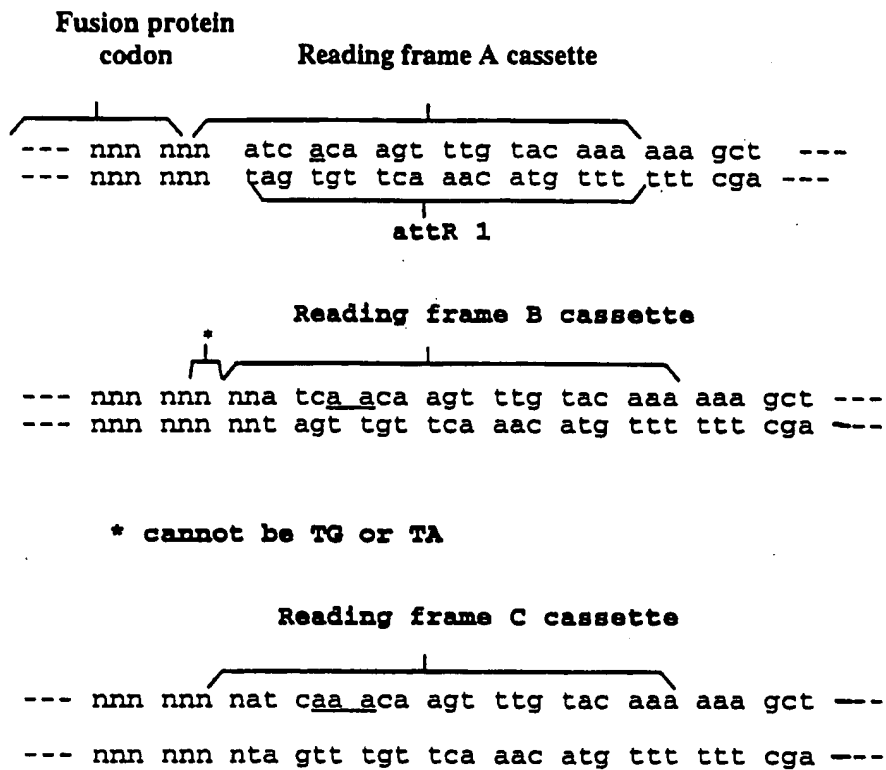


FIGURE 79



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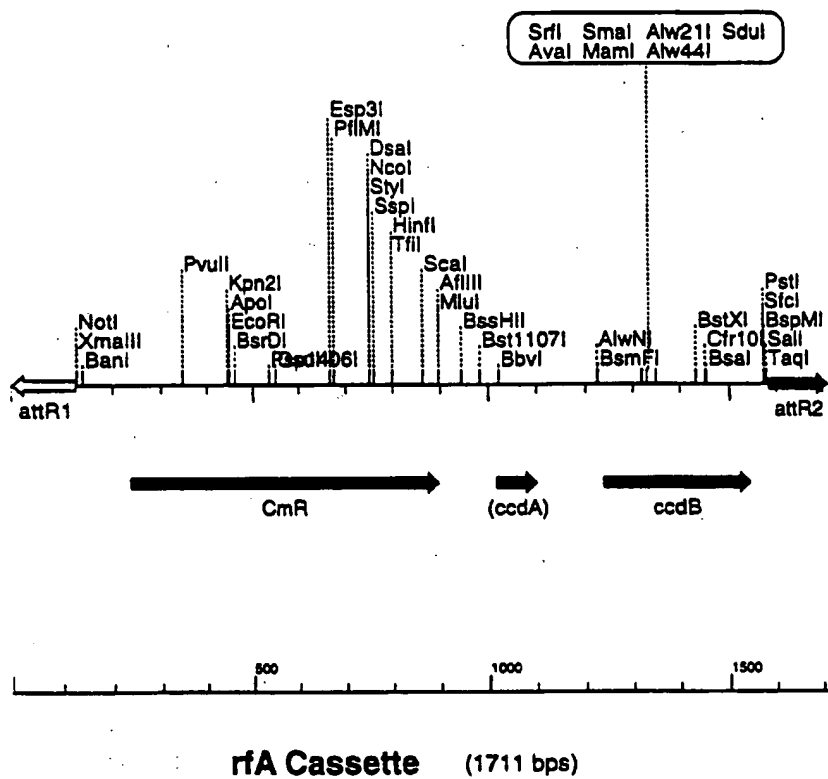


FIGURE 80

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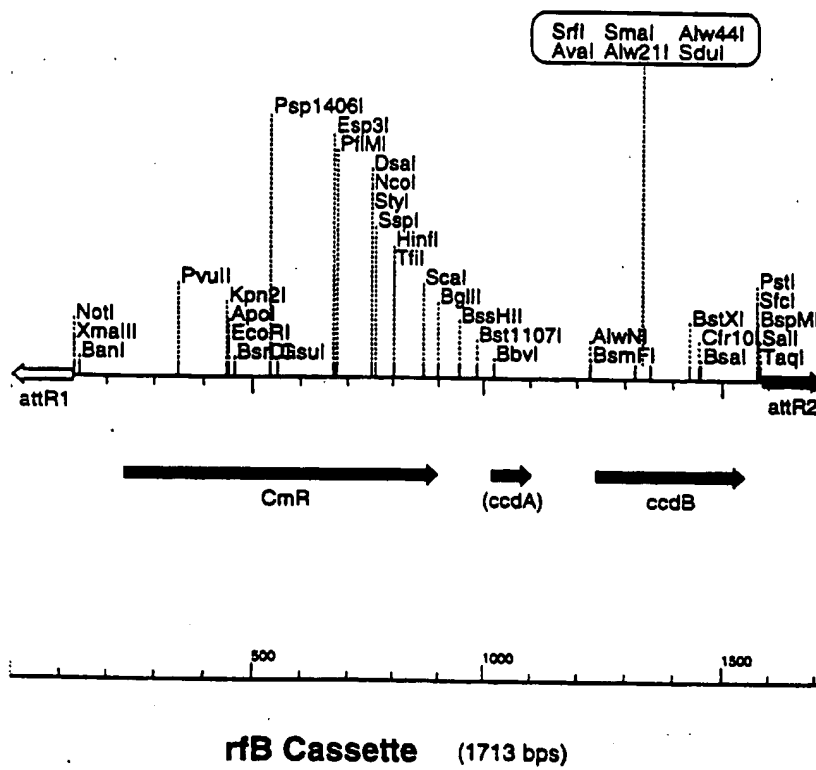
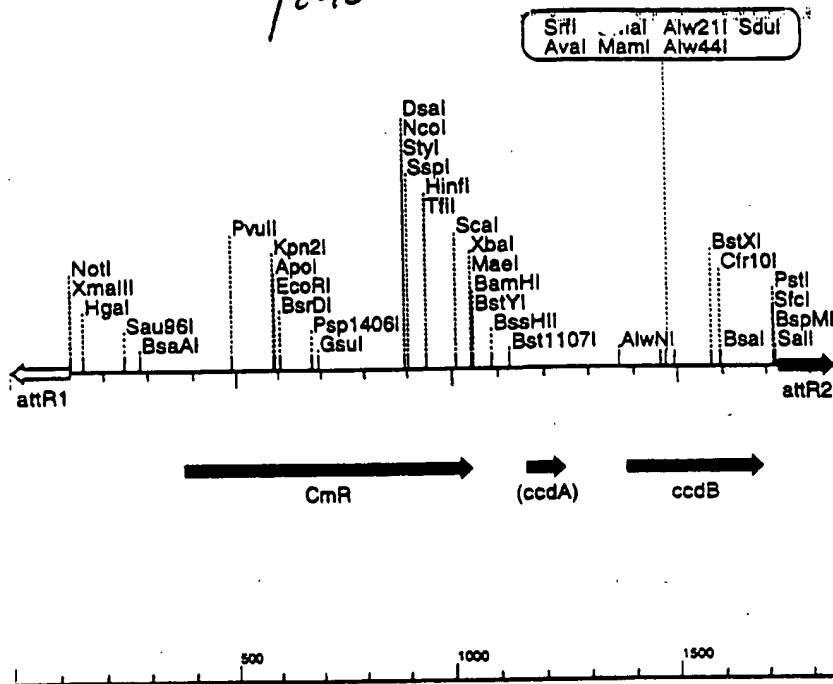


FIGURE 81

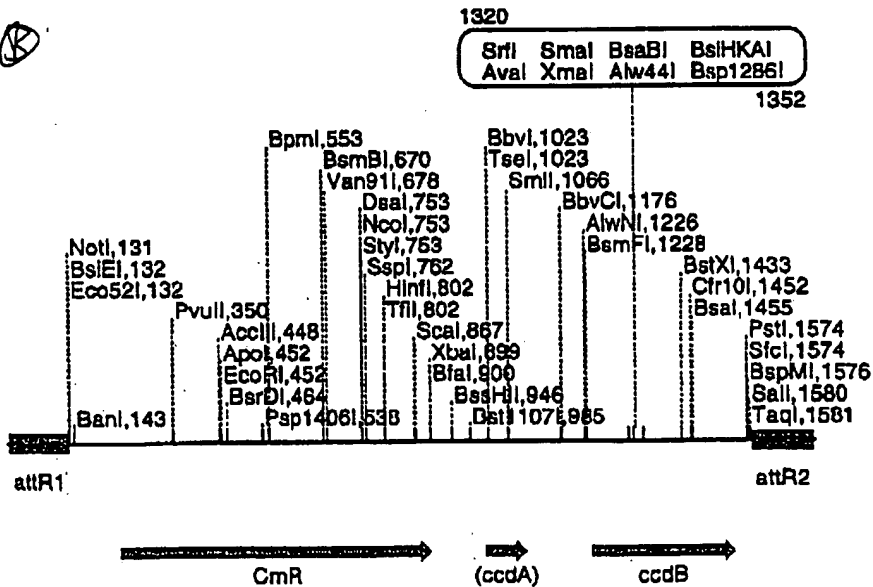
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(A)



rC Cassette (1856 bps)

(B)



rC cassette (1715 bps)

FIGURE 82

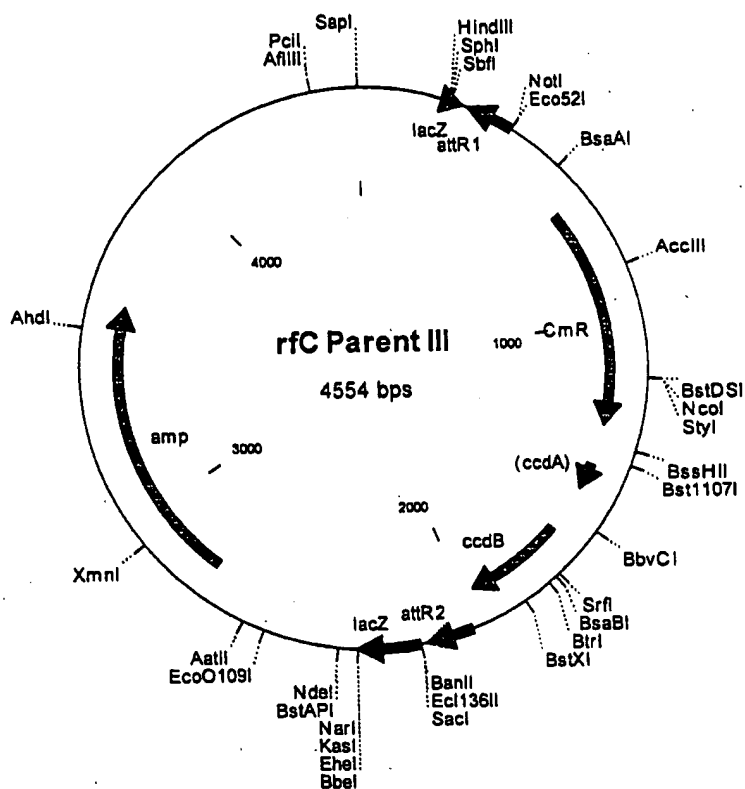


FIGURE 83A

## prfC Parent III 4554 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
410..286		attR1
660..1319		CmR
1439..1523		inactivated ccdA
1661..1966		ccdB
2007..2131		attR2
2753..3613		amp

1	GCGCCCAATA	CGCAAACCGC	CTCTCCCCGC	GCGTTGGCCG	ATTCATTAAT	GCAGCTGGCA
61	CGACAGGTTT	CCCGACTGGA	AAGCGGGCAG	TGAGCGCAAC	GCAATTAATG	TGAGTTAGCT
121	CACTCATTAG	GCACCCCAGG	CTTTACACTT	TATGCTTCCG	GCTCGTATGT	TGTGTGGAAT
181	TGTGAGCGGA	TAACAATTTT	ACACAGGAAA	CAGCTATGAC	CATGATTACG	CCAAGCTTGC
241	ATGCCTGCAG	GTCGACTCTA	GAGGATCCCC	GGGTACCGAT	ATCAAACAAG	TTTGACAAA
301	AAAGCTGAAC	GAGAAACGTA	AAATGATATA	AATATCAATA	TATTAAATTA	GATTTTGCAT
361	AAAAACAGA	CTACATAATA	CTGTAAAACA	CAACATATCC	AGTCACTATG	GCGGCCGCTA
421	AGTTGGCAGC	ATCACCCGAC	GCACTTTGCG	CCGAATAAAT	ACCTGTGACG	GAAGATCACT
481	TCGCAGAATA	AATAAATCCT	GGTGTCCCTG	TTGATACCGG	GAAGCCCTGG	GCCAACCTTT
541	GGCGAAAATG	AGACGTTGAT	CGGCACGTAA	GAGGTTCCAA	CTTTCACCAT	AATGAAATAA
601	GATCACTACC	GGGCGTATTT	TTTGAGTTAT	CGAGATTTTC	AGGAGCTAAG	GAAGCTAAAA
661	TGGAGAAAAA	AATCACTGGA	TATACCACCG	TTGATATATC	CCAATGGCAT	CGTAAAGAAC
721	ATTTTGAGGC	ATTTCACTCA	GTTGCTCAAT	GTACCTATAA	CCAGACCGTT	CAGCTGGATA
781	TTACGGCCTT	TTTAAAGACC	GTAAGAAAAA	ATAAGCACAA	GTTTTATCCG	GCCTTTATTC
841	ACATTCTTGC	CCGCCTGATG	AATGCTCATC	CGGAATTCCG	TATGGCAATG	AAAGACGGTG
901	AGCTGGTGAT	ATGGGATAGT	GTTACCCCTT	GTTACACCGT	TTTCCATGAG	CAAACTGAAA
961	CGTTTTTCATC	GCTCTGGAGT	GAATACCACG	ACGATTTCCT	GCAGTTTCTA	CACATATATT
1021	CGCAAGATGT	GGCGTGTTAC	GGTGAAAACC	TGGCCTATTT	CCCTAAAGGG	TTTATTGAGA
1081	ATATGTTTTT	CGTCTCAGCC	AATCCCTGGG	TGAGTTTCAC	CAGTTTGTAT	TTAAACGTGG
1141	CCAATATGGA	CAACTTCTTC	GCCCCCGTTT	TCACCATGGG	CAAAATATTAT	ACGCAAGGCG
1201	ACAAGGTGCT	GATGCCGCTG	GCGATTCCAG	TTCATCATGC	CGTCTGTGAT	GGCTTCCATG
1261	TCGGCAGAAT	GCTTAATGAA	TTACAACAGT	ACTGCGATGA	GTGGCAGGGC	GGGGCGTAAT
1321	CTAGAGGATC	CGGCTTACTA	AAAGCCAGAT	AACAGTATGC	GTATTTGCGC	GCTGATTTTT
1381	GCGGTATAAG	AATATATACT	GATATGTATA	CCCGAAGTAT	GTCAAAAAGA	GGTGTGCTAT
1441	GAAGCAGCGT	ATTACAGTGA	CAGTTGACAG	CGACAGCTAT	CAGTTGCTCA	AGGCATATAT
1501	GATGTCAATA	TCTCCGGTCT	GGTAAGCACA	ACCATGCAGA	ATGAAGCCCC	TCGTCTCGCT
1561	GCCGAACGCT	GGAAAGCGGA	AAATCAGGAA	GGGATGGCTG	AGGTCGCCCG	GTTTATTGAA
1621	ATGAACGGCT	CTTTTGCTGA	CGAGAACAGG	GACTGGTGAA	ATGCAGTTTA	AGGTTTACAC
1681	CTATAAAAGA	GAGAGCCGTT	ATCGTCTGTT	TGTGGATGTA	CAGAGTGATA	TTATTGACAC
1741	GCCCCGGCGA	CGGATGGTGA	TCCCCCTGGC	CAGTGCACGT	CTGCTGTCAG	ATAAAGTCTC
1801	CCGTGAACCT	TACCCGGTGG	TGCATATCGG	GGATGAAAGC	TGGCGCATGA	TGACCACCGA
1861	TATGGCCAGT	GTGCCGGTCT	CCGTTATCGG	GGAAGAAGTG	GCTGATCTCA	GCCACCGCGA
1921	AAATGACATC	AAAAACGCCA	TTAACCTGAT	GTTCTGGGGA	ATATAAATGT	CAGGCTCCGT
1981	TATACACAGC	CAGTCTGCAG	GTGACCCATA	GTGACTGGAT	ATGTTGTGTT	TTACAGTATT
2041	ATGTAGTCTG	TTTTTTATGC	AAAATCTAAT	TTAATATATT	GATATTTATA	TCATTTTACG
2101	TTTCTCGTTC	AGCTTTCTTG	TACAAAGTGG	TTCGATATCG	GTACCGAGCT	CGAATTCACT
2161	GGCCGTCGTT	TTACAACGTC	GTGACTGGGA	AAACCCTGGC	GTTACCCAAC	TTAATCGCCT
2221	TGCAGCACAT	CCCCCTTTTG	CCAGCTGGCG	TAATAGCGAA	GAGGCCCGCA	CCGATCGCCC
2281	TTCCCAACAG	TTGCGCAGCC	TGAATGGCGA	ATGGCGCCTG	ATGCGGTATT	TTCTCCTTAC
2341	GCATCTGTGC	GGTATTTTAC	ACCGCATATG	GTGCACTCTC	AGTACAATCT	GCTCTGATGC
2401	CGCATAGTTA	AGCCAGCCCC	GACACCCGCC	AACACCCGCT	GACGCGCCCT	GACGGGCTTG
2461	TCTGCTCCCG	GCATCCGCTT	ACAGACAAGC	TGTGACCGTC	TCCGGGAGCT	GCATGTGTCA
2521	GAGGTTTTCA	CCGTCTATCA	CGAAACGCGC	GAGACGAAAG	GGCCTCGTGA	TACGCCTATT
2581	TTTATAGGTT	AATGTCATGA	TAATAATGGT	TTCTTAGACG	TCAGGTGGCA	CTTTTCGGGG
2641	AAATGTGCGC	GGAACCCCTA	TTTGTTTATT	TTTCTAAATA	CATTCAAATA	TGTATCCGCT
2701	CATGAGACAA	TAACCCTGAT	AAATGCTTCA	ATAATATTGA	AAAAGGAAGA	GTATGAGTAT
2761	TCAACATTTT	CGTGTGCCCC	TTATTCCCTT	TTTTCGGGCA	TTTTGCCTTC	CTGTTTTTGC -

2821 TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG  
2881 TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTTCGCC CCGAAGAACG  
2941 TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTATTGA  
3001 CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT CAGAATGACT TGGTTGAGTA  
3061 CTCACCACTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC  
3121 TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC  
3181 GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACCTGCC TTGATCGTTG  
3241 GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCTGTAGC  
3301 AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA  
3361 ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT  
3421 TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT  
3481 CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG  
3541 GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT  
3601 TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAACT  
3661 TCATTTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAT  
3721 CCTTAACGT GAGTTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC  
3781 TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT  
3841 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTCCGA AGGTAACCTG  
3901 CTTCAGCAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA  
3961 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC  
4021 TGCTGCCAGT GCGGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA  
4081 TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TCGGTGCACA CAGCCCAGCT TGGAGCGAAC  
4141 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA  
4201 AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG  
4261 GGAGCTTCCA GGGGGAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG  
4321 ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG  
4381 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC  
4441 TGCCTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC  
4501 TCGCCGACG CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGA

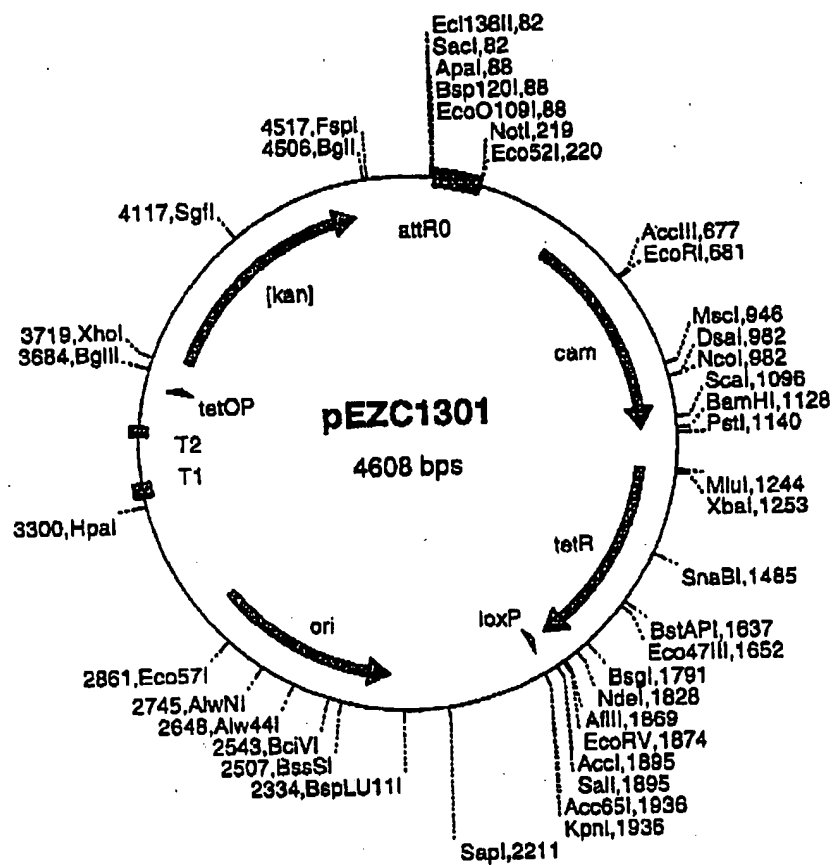


FIGURE 84

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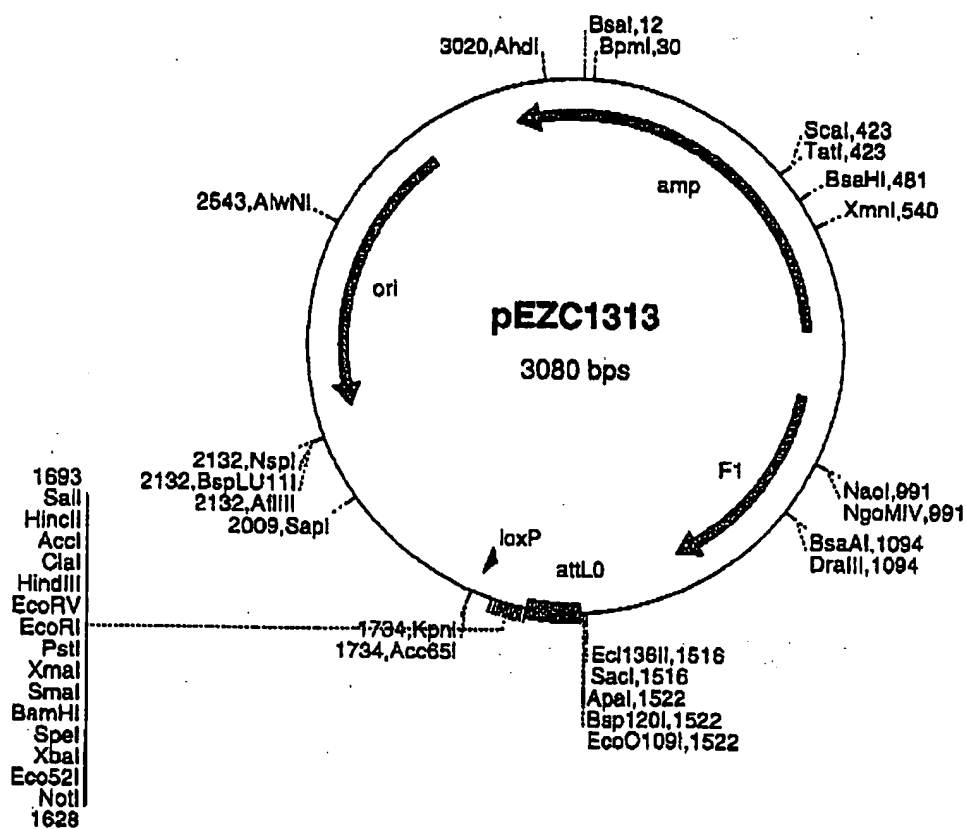


FIGURE 85



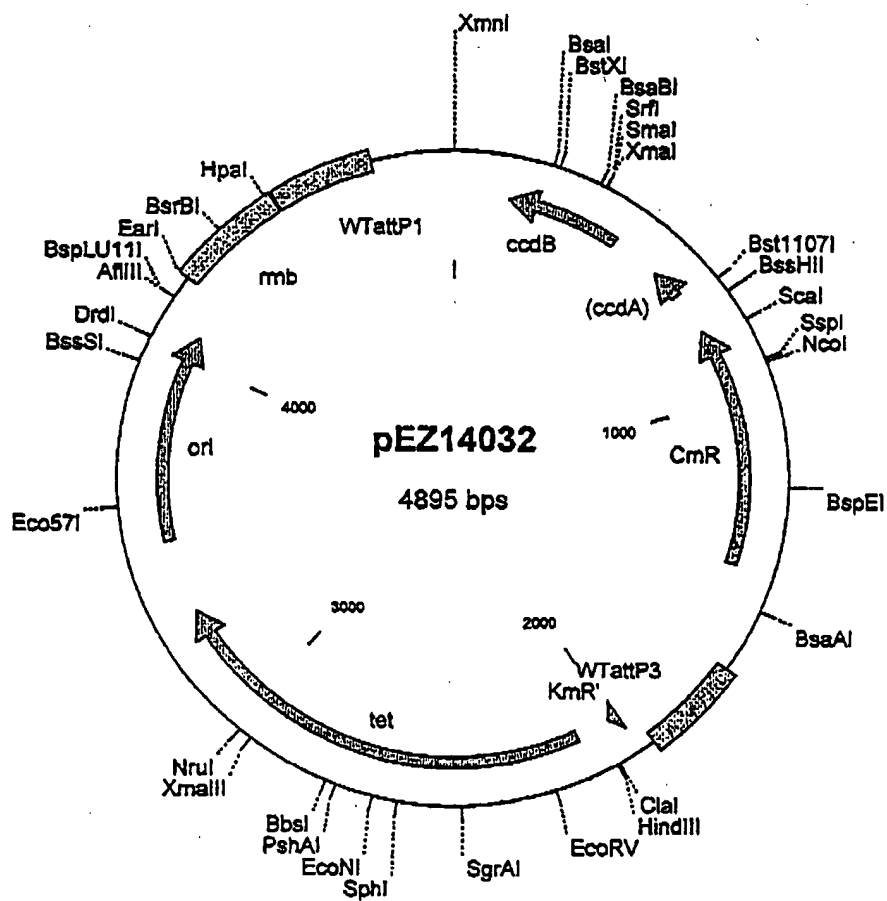


FIGURE 86

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FIGURE 87

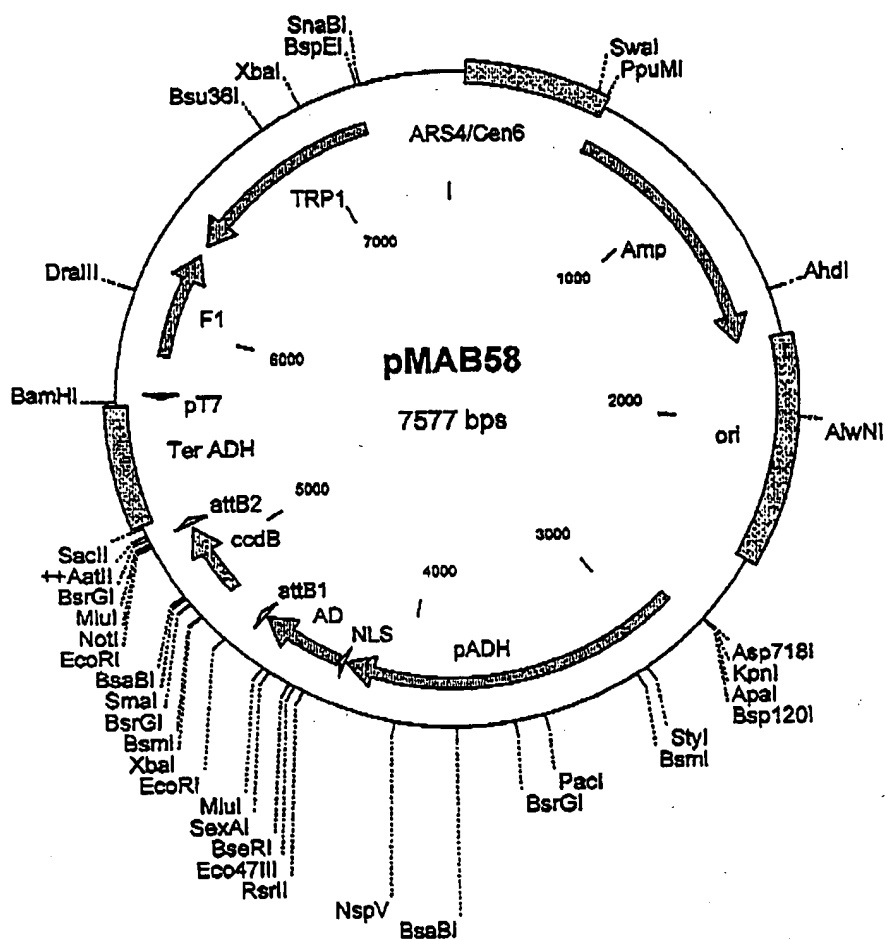
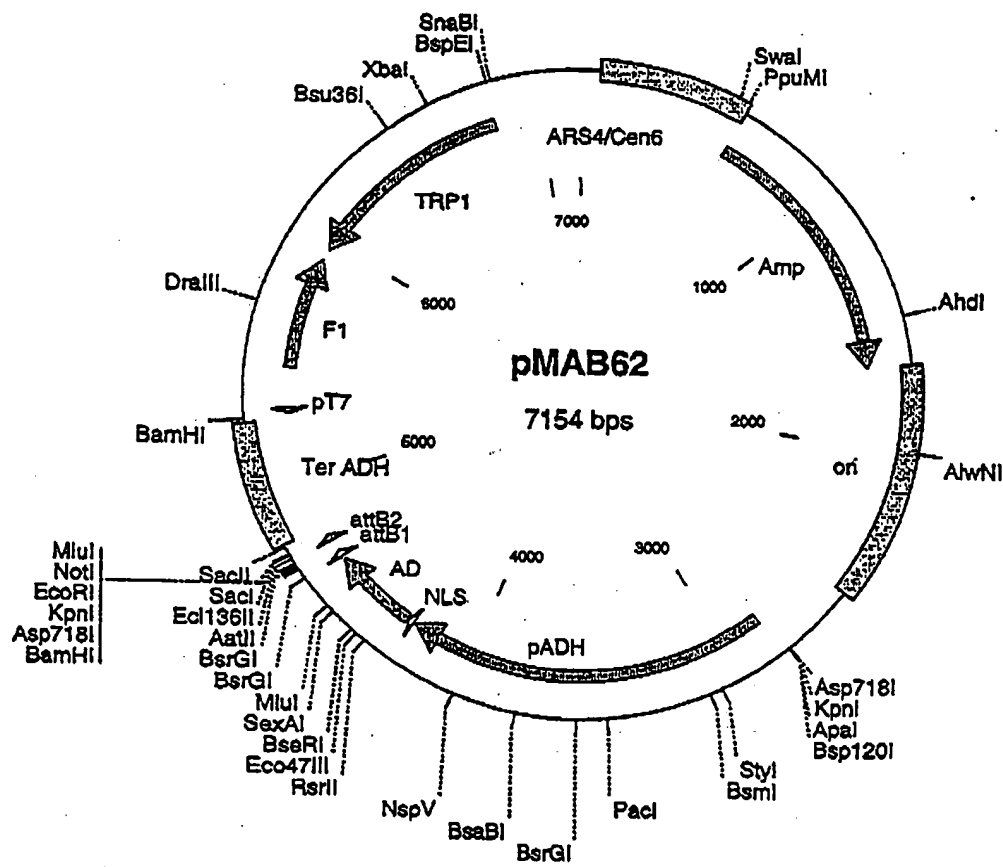
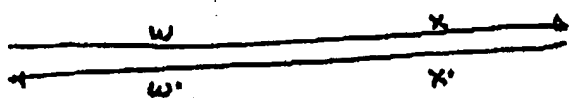


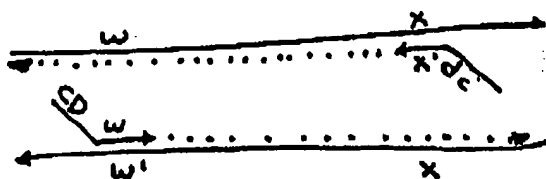
FIGURE 88



DNA to be amplified (5' → 3'):



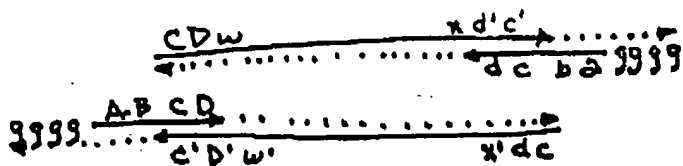
↓ Denature, anneal  
hybrid primers,  
↓ extend with polymerase



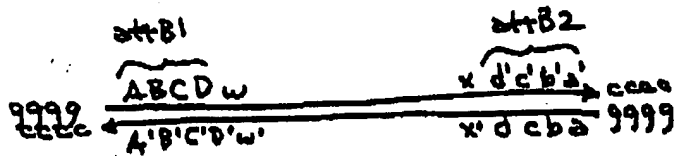
↓ amplification cycles



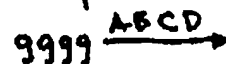
↓ Denature, anneal  
attB primers,  
extend with polymerase



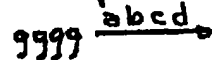
↓ amplification cycles



attB1 primer:



attB2 primer:



Hybrid primers (part  
attB, part gene  
specific):



FIGURE 89

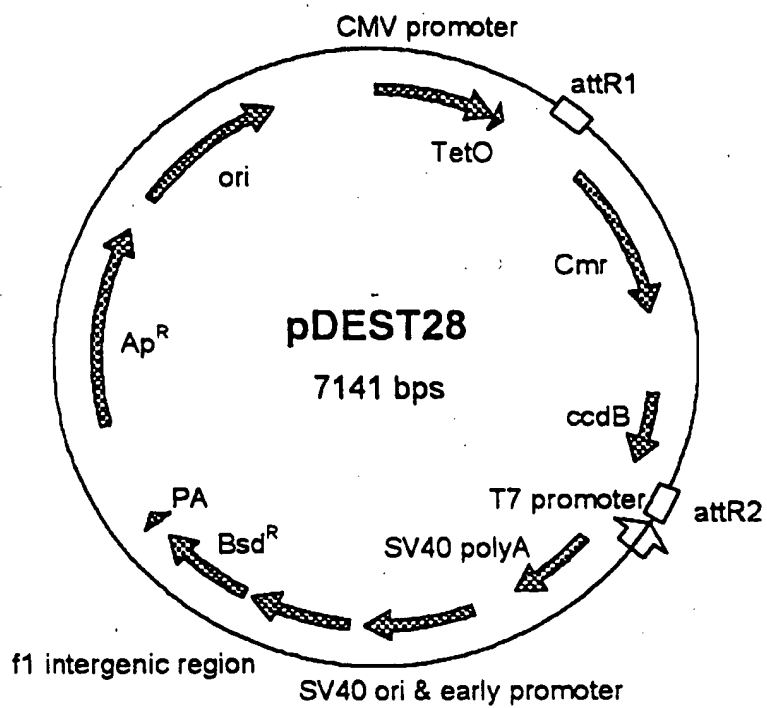


FIGURE 90A

pDEST28 7141 bp

ATGCATGTCGTTACATAA CTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCC  
CGCCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGTATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGTTTTGAC  
TCACGGGGATTTC AAGTCTCCACCCCATTGACGTCAATGGGAGTTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCTGAACAACCTCCGCCCATTTGACGCAATGGGCGGT  
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGATTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACT  
CTAGAGGATCCCTACCGGTGATATCCTCGAGCCCATCAACAAGTTTGTACAAAAAAGCTG  
AACGAGAAACGTAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAAC  
AGACTACATAATACTGTAAACACAACATATCCAGTCACTATGGCGGCCGCATTAGGCAC  
CCCAGGCTTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGTAGTTAGGATCC  
GGCGAGATTTT CAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCAC  
CGTTGATATGATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGGAGA  
ATGTACCTATAACAGCAGCCGTTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAA  
AAATAAGCACAAAGTTTATCCGGCCTTTATTTCACATTTCTGCCCCGCTGATGAATGCTCA  
TCCGGAATTCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCC  
TTGTTACACCGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCA  
CGACGATTTCCGGCAGTTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAA  
CCTGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTG  
GGTGAGTTTTACCAAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGT  
TTTTACCATTGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCA  
GGTTCATCATGCCGTCTGTGATGGCTTCCATGTGCGGCAGAATGCTTAATGAATTACAACA  
GTACTGCGATGAGTGGCAGGGCGGGGCGTAAAGATCTGGATCCGGCTTACTAAAAGCCAG  
ATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTA  
TACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGAC  
AGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCA  
CAACCATGCAGAATGAAGCCCGTCGTCTCGTGCCGAACGCTGGAAAGCGGAAAAACAG  
AAGGACTGGCTGAGGTGCGCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACA  
GGGACTGGTGAAATGCAGTTTAAGGTTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTG  
TTTGTGGATGTACAGAGTGATATTATTGACACGCCCGGGCGACGGATGGTGATCCCCCTG  
GCCAGTGCACGTCTGCTGTGAGATAAAGTCTCCCGTGAACCTTACCCGGTGGTGATATC  
GGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTCGGGTCTCCGTTATC  
GGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACTG  
ATGTTCTGGGGAATATAAATGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTGACCA  
TAGTGACTGGATATGTTGTGTTTTACAGTATTATGATAGTCTGTTTTTTATGCAAAATCTA  
ATTTAATATATTGATATTTATATCATTTTTACGTTTTCTCGTTCAGCTTTCTTGTACAAAGT  
GGTTGATGGGCGGCGCTCTAGAGGGCCCAAGCTTACGCGTGATGCGACGTCATAGCTC  
TCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTCGTTTTACAACGTCGTGA  
CTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATA  
ATTGGACAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTAAGTGT  
ATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTTGCTTACTGAGTATGA  
TTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTGTGTATTTTAGATTCA  
CAGTCCCAAGGCTCATTTTCAGGCCCTCAGTCCTCACAGTCTGTTTCATGATCATAATCAG  
CCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTGAA  
CCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTTATTGCAGCTTATAATGG  
TTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTCACTGCATT  
TAGTTGTGGTTTTGTCCAACTCATCAATGTATCTTATCATGTCTGGATCGATCCTGCAAT  
AATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGCTGGCGTAATAGCGAAG  
AGGCCGCGACCGATCGCCCTTCCCAACAGGCTGCGCAGCCTGAATGGCGAATGGGACGCGC  
CCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACAC  
TTGCCAGCGCCCTAGCGCCCGCTCCTTTGCTTTCTTCCCTTCTTCTCGCCACGTTCCG  
CCGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTT-

Figure 90B

TACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGC  
CCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCT  
TGTTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGA  
TTTTGCCGATTTTCGGCCTATTGGTTAAAAATGAGCTGATTAAACAAATATTTAACGCGA  
ATTTTAACAAAATATTAACGTTTACAATTTTCGCCTGATGCGGTATTTTCTCCTTACGCAT  
CTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCATGGCCTGAAATAACCT  
CTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGT  
GTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGC  
ATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTA  
TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCCATCC  
CGCCCCCTAACTCCGCCCCAGTTCGCCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTA  
TTTTATGCAGAGGCCGAGGCCGCTCGGCCCTGAGCTATTCCAGAAGTAGTGAGGAGGCT  
TTTTTGGAGGCCCTAGGCTTTTGCAAAAGCTTGATTCTTCTGACACAACAGTCTCGAAGCT  
TAAGACCATGGCCAAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAACGGC  
TACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAG  
CGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTTACTGGGGGACCTTGTGCAGA  
ACTCGTGGTGCTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGC  
GATCGGAAATGAGAACAGGGGCATCTTGAGCCCCCTGCGGACGGTGCCGACAGGTGCTTCT  
CGATCTGCATCTCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACGGCAGT  
TGGGATTCGTGAATTTGCTGCCCCCTGCGTTATGTGTGGGAGGGCTAAGCACTTCGTGGCCG  
AGTTTCGAAATGACCGACCAAGCGACGCCCCAACCTGCCATCACGATGGCCGCAATAAAATA  
TCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGATAGCGATAAGGATC  
CGCGTATGGTGCATCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGA  
CACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTAC  
AGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTCCCGTCATCACCG  
AAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTATGATA  
ATAATGGTTTCTTAGACGTGAGGTGGCACTTTTTCGGGGAAATGTGCGCGGAACCCCTATT  
TGTTTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAA  
ATGCTTCAATAATATTGAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCCCCCTT  
ATTCCCTTTTTTGCGGCATTTTGCCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAA  
GTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAAC  
AGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTTCCAATGATGAGCACTTTT  
AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGT  
CGCCGCATACACTATTCTCAGAATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAC  
CTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAC  
ACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTG  
CACAACATGGGGGATCATGTAACCTGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCC  
ATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAA  
CTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAG  
GCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCT  
GATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGAT  
GGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAA  
CGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGAC  
CAAGTTTACTCATATATACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAGGATC  
TAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTT  
CACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTG  
CGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCG  
GATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCA  
AATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCCTTCAAGAACTCTGTAGCACCG  
CCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTG  
TGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGGCTGA  
ACGGGGGGTTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATAC  
CTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTAT  
CCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCC  
TGCTATCTTTATAGTCTGCTGCGGTTTCCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA  
TGCTCGTCAGGGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGCCCTTTTACGGTTT  
CTGGCCTTTTTGCTGGCCTTTTTGCTCACATGTTCTTTCTGCGTTATCCCCTGATTCTGTG  
GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACCCGAACGACCGAG-

FIGURE 90C

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CGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCC  
GCGCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGA  
AGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTAGAAAAAT  
AAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACC  
ATTATTATCATGACATTAACCTATAAAAAATAGGCGTAGTACGAGGCCCTTCACTCATTA  
G

FIGURE 90D



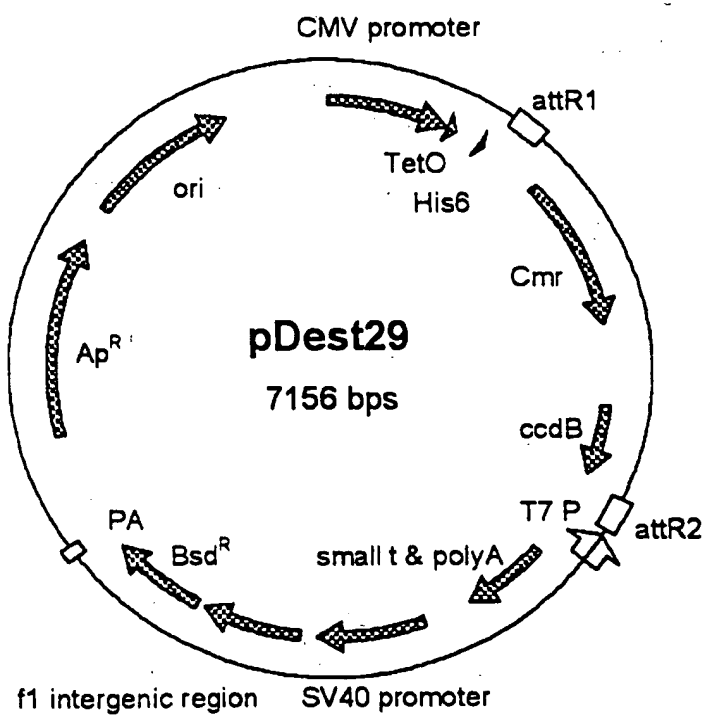


FIGURE 91 A

pDEST29 7156 bp

ATGCATGTGCTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCC  
CGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTTCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGAC  
TCACGGGGATTTC AAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGT TTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAATGGGCGGT  
AGGCGTGATCGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTTCGACGAGCTCGTTTAGTGAAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACC  
ATGGCGTACTACCATCACCATCACCATCACACCGGTGATATCCTCGAGCCCATCACAAGT  
TTGTACAAAAAAGCTGAACGAGAAACGTAATATGATATAAATATCAATATATTAATTAG  
ATTTTGCATAAAAAACAGACTACATAACTGTAAACACACATATCCAGTCACTATGG  
CGGCCGATTAGGCACCCCAAGGCTTTACAGCTTTATGCTTCCGGCTCGTATAATGTGTGGA  
TTTTGAGTTAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAAA  
TCACTGGATATACACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCAT  
TTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTT  
TAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTACATTCTTGCCC  
GCCTGATGAATGCTCATCCGGAATTCGATATGGCAATGAAAGACGGTGAGCTGGTGATAT  
GGGATAGTGTTTCAACCTTGTTACACCGTTTTCCATGAGCAAACCTGAAACGTTTTCATCGC  
TCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTGCAAGATGTGG  
CGTGTTACGGTGAAAACTGGCCTATTTCCCTAAAGGTTTATTGAGAATATGTTTTTCG  
TCTCAGCCAAATCCCTGGGTGAGTTTACACAGTTTTGATTAAACGTGGCCAATATGGACA  
ACTTCTTCGCCCCCGTTTTACCATGGGCAATATTATACGCAAGGCGACAAGGTGCTGA  
TGCCGCTGGCGATT CAGGTTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAAATGC  
TTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCGTAAACCGCTGGATCCG  
GCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAA  
TATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTAT  
TACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATC  
TCCGGTCTGGTAAGCACAACCATGCAGATGAAGCCCGTCTGCTGCGTGCCGAACGCTGG  
AAAGCGGAAATCAGGAAGGGATGGCTGAGGTGCGCCCGGTTTATTGAAATGAACGGCTCT  
TTTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGA  
GAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACG  
GATGGTGATCCCCCTGGCCAGTGCACGTCTGCTGTCAGATAAAGTCTCCCGTGAACCTTTA  
CCCCGTGGTGATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGT  
GCCGTTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAAATGACATCAA  
AAACGCCATTAACTGATGTTTCTGGGGAATATAAATGT CAGGCTCCGTTATACACAGCCA  
GTCTGCAGGTGACCATAGTGAAGTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTT  
TTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTT CAG  
CTTTCTTGATACAAAGTGGTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTG CAT  
GCGACGT CATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTGCT  
TTTACAACGTGCTGACTGGGAAAACTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTT  
CTGTGGTGTGACATAATTGGACAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATAT  
AAAATTTTTAAGTGATAATGTGTTAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTT  
GCTTACTGAGTATGATTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTG  
TGTATTTTAGATT CACAGTCCCAAGGCTCATTT CAGGCCCCCTCAGTCCTCACAGTCTGTT  
CATGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCC  
ACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTAT  
TGCAGCTTATAATGGTTACAAATAAGCAATAGCATCACAAATTTCAAAATAAAGCATT  
TTTTTCACTGCATTCTAGTTGTGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTG  
GATCGATCCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGCT  
GGCGTAATAGCGAAGAGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATG  
GCGAATGGGACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTTGGTTACGCGCA  
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTGCTTTCTTCCCTTCTT  
TTCTCGCCACGTTTCGCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT-

FIGURE 91B

TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCAC  
GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT  
TTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTT  
TTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAAC  
AAATATTTAACGCGAATTTTAAACAAAATATTAAACGTTTACAATTTTCGCCTGATGCGGTAT  
TTTCTCCTTACGCATCTGTGCGGTATTTACACCGCATACGCGGATCTGCGCAGCACCAT  
GGCCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCGGAAAGAACC  
AGCTGTGGAATGTGTGTCTAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAA  
GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCC  
CAGCAGGCAGAAATATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCC  
TAACTCCGCCCCATCCCGCCCCCTAACTCCGCCCCATTCTCCGCCCCATGGCT  
GACTAATTTTTTTTATTATTTATGAGAGGCCGAGGCCCTCGGCCTCTGAGCTATTCCAGA  
AGTAGTGAGGAGCTTTTTTGGAGGCCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACA  
CAACAGTCTCGAACTTAAGACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCAT  
TGAAAGAGCAACGGCTACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTTCGCCAG  
CGCAGCTCTCTCTAGCGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTTACTGG  
GGGACCTTGTGCGAACTCGTGGTGCTGGGCACCTGCTGCTGCTGCGGCAGCTGGCAACCT  
GACTTGTATCGTCGCGATCGGAAATGAGAACAGGGGCATCTTGAGCCCCCTGCGGACGGTG  
CCGACAGGTGCTTCTCGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGG  
ACAGCCGACGGCAGTTGGGATTCTGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTA  
AGCACTTCGTGGCCGAGTTGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAT  
GGCCGCAATAAAATATCTTTATTTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCG  
ATAGCGATAAGGATCCGCGTATGGTGCATCTCTCAGTACAATCTGCTCTGATGCCGCATAG  
TTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTC  
CCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCTAGAGGTTT  
TCACCGTCATCACGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAG  
GTTAATGTCTATGATAAATAGTTTTCTTAGACGTGAGGTGGCACTTTTCGGGGAAATGTG  
CGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGA  
CAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACAT  
TTCCGTGTGCGCCCTTATTCCTTTTTTTGCGGCATTTTGCCCTTCTGTTTTTGTCTACCCA  
GAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATC  
GAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCA  
ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGG  
CAAGAGCAACTCGGTCCGCCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCA  
GTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATA  
ACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAG  
CTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCG  
GAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCA  
ACAACGTTGCGCAAACTATTAAGTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTA  
ATAGACTGGATGGAGGCGGATAAAGTTGAGGACCACTTCTGCGCTCGGCCCTTCCGGCT  
GGCTGGTTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCA  
GCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAG  
GCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAAGCAT  
TGGTAACGTGACAGCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTT  
TAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAA  
CGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGA  
GATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACCACCGCTACCAGCG  
GTGGTTTTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGC  
AGAGCGCAGATACCAAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAG  
AACTCTGTAGCACCGCCTACATACTCTGCTGCTAATCCTGTTACCAAGTGGCTGCTGCC  
AGTGGCGATAAGTCTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCG  
CAGCGGTCCGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGAGCGAACGACCTAC  
ACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGA  
AAGGCGGACAGGTATCCGGTAAGCGGCGAGGTGCGAACAGGAGAGCGCACGAGGGAGCTT  
CCAGGGGGAACCGCCTGGTATCTTTATAGTCTGTGCGGTTTTCGCCACCTCTGACTTGAG  
CGTCTGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCG  
GCCTTTTTACGGTTCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCGTTA  
TCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGC-

AGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGC  
AAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTT  
TTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAA  
TGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCT  
GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTAGTACGAGG  
CCCTTTCACCTCATTAG

FIGURE 91D

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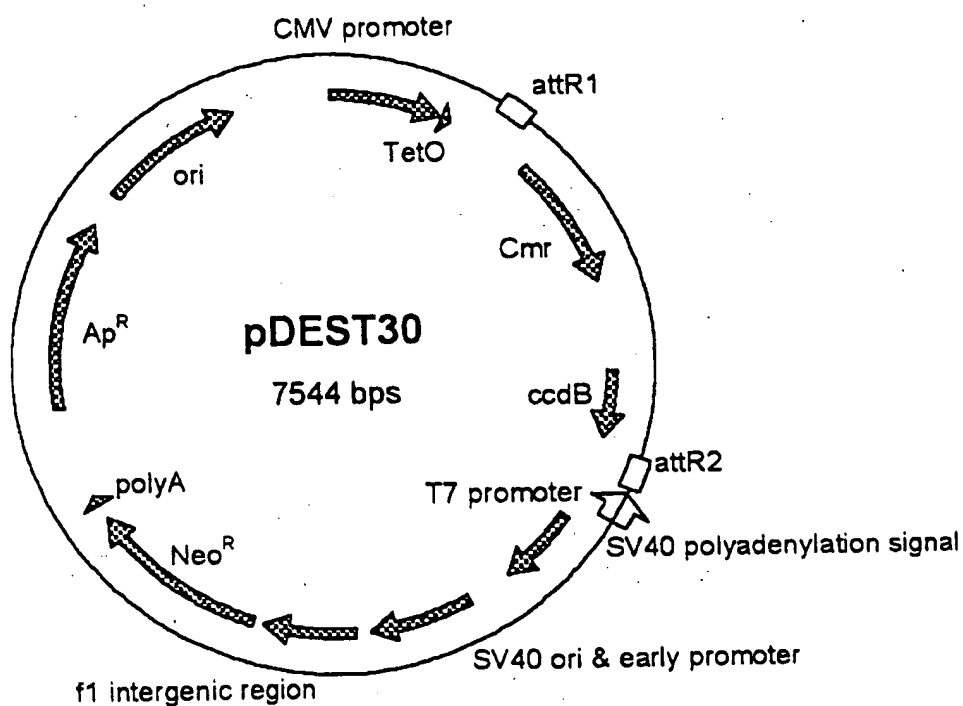


FIGURE 92A

pDEST30 7544 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCC  
CGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGTATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGAC  
TCACGGGGATTTCCAAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCTGAACAACTCCGCCCCATTGACGCAAATGGGCGGT  
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACT  
CTAGAGGATCCCTACCGGTGATATCCTCGAGCCCATCAACAAGTTTGTACAAAAAGCTG  
AACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAAC  
AGACTACATAATACGTGTAACACACACATATCCAGTCACTATGGCGGCCGATTAGGCAC  
CCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGTAGTTAGGATCC  
GGCGAGATTTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCAC  
CGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGTAGGCATTTTCAGTCAGTTGCTCA  
ATGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAA  
AAATAAGCACAAAGTTTTATCCGGCCTTTATTACATTCCTGCCCCCTGATGAATGCTCA  
TCCGGAATTCGGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCC  
TTGTTACACCGTTTTTCCATGAGCAAACTGAAACGTTTTTCATCGCTCTGGAGTGAATACCA  
CGACGATTTCCGGCAGTTTCTACACATATATTGCAAGATGTGGCGTGTACGGTGAAAA  
CCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTG  
GGTGAGTTTACCAGTTTGTATTTAAACGTGGCCAATATGGACAACCTCTTCCGCCCCGT  
TTTACCATGGGCAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCA  
GGTTCATCATGCCGTCTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACA  
GTACTGCGATGAGTGGCAGGGCGGGCGTAAAGATCTGGATCCGGCTTACTAAAAGCCAG  
ATAACAGTATGCGTATTTTGGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTA  
TACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGAC  
AGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCA  
CAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAATCAGG  
AAGGGATGGCTGAGGTGCGCCCGGTTTATTGAAATGAACGGCTCTTTTGCTGACGAGACA  
GGGACTGGTGAAATGCAGTTTAAAGTTTACACCTATAAAAAGAGAGCCGTTATCGTCTG  
TTTGTGGATGTACAGAGTGATATTATTGACACGCGCCGCGGCGACGGATGGTGATCCCCCTG  
GCCAGTGCACGTCTGCTGTGATGATAAGTCTCCCGTGAACCTTACC CGGTGGTGATATC  
GGGGATGAAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATC  
GGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACTG  
ATGTTCTGGGGAATATAAATGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTGACCA  
TAGTGAAGTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTA  
ATTTAATATATTGATATTTATATCATTTTACGTTTTCTCGTTCAGCTTTCTTGTAACAAGT  
GGTTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGCATGCGACGTATAGCTC  
TCTCCCTATAGTGAGTGTGATTATAAGCTAGGCACTGGCCGTGTTTTACAACGTGCTGA  
CTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATA  
ATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTAAGTGT  
ATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTTGCTTACTGAGTATGA  
TTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTGTGTATTTTAGATTCA  
CAGTCCCCAAGGCTCATTTTCAGGCCCCCTCAGTCTCAGATCTGTTTCATGATCATAATCAG  
CCATACCACATTTGTAGAGGTTTTACTTGTCTTTAAAAAACCTCCACACCTCCCCCTGAA  
CCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTTATTGCAGCTTATAATGG  
TTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTCAGTGCATT  
TAGTTGTGGTTTTGTCCAACTCATCAATGTATCTTATCATGTCTGGATCGATCCTGCATT  
AATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGCTGGCGTAATAGCGAAG  
AGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGC  
CCTGTAGCGGCGCATTAAAGCGCGGCGGGTGGTGGTTACGCGCAGCGTGACCGCTACAC  
TTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTCCCTTCTTCTCGCCACGTTTCG  
CCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCGGATTTAGTGCTT-

FIGURE 92B

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TACGGCACCTCGACCCCCAAAAAAGTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGC  
CCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCT  
TGTTCCAAACTGGAACAACACTCAACCCCTATCTCGGTCTATTCTTTTGATTTATAAGGGA  
TTTTGCCGATTTCCGGCCTATTGGTTAAAAAATGAGCTGATTAAACAAATATTTAACCGCA  
ATTTTAACAAATATTAACGTTTACAATTTTCGCCTGATGCGGTATTTCTCCTTACGCAT  
CTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCATGGCCTGAAATAACCT  
CTGAAAGAGGAACCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACAGCTGTGGAATGTGT  
GTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGC  
ATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTA  
TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCCGCCCTAACTCCGCCCATCC  
CGCCCCTAACCTCCGCCCAGTTCCGCCCATTTCTCGCCCCATGGCTGACTAATTTTTTTTA  
TTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCAGAAGTAGTGAGGAGGCT  
TTTTTGAGGGCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACACAACAGTCTCGAACT  
TAAGGCTAGAGCCACCATGATTGAACAAGATGGATTGCACGCAGGTTCTCGGCCGCTTG  
GGTGGAGAGGCTATTCCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGC  
CGTGTTCGGCTGTGACGCGAGGGGCGCCCGGTTCTTTTGTCAAGACCGACCTGTCCGG  
TGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGT  
TCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGG  
CGAAGTGCCGGGGCAGGATCTCCTGTCTATCTACCTTGCTCCTGCCGAGAAAGTATCCAT  
CATGGCTGATGCAATCGCGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCA  
CCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGGTCTTGTGATCA  
GGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGTGTTCGCCAGGCTCAA  
GGCGCGCATGCCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCCTGCTTGCCGAA  
TATCATGGTGGAAAATGGCCGCTTTTCTGGATTCTCGACTGTGGCCGGCTGGGTGTGGC  
GGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGCGCGCA  
ATGGGCTGACCGCTTCTCCTGCTTTACGGTATCGCCGCTCCCGATTCCGACGCGCATCGC  
CTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGACTCTGGGGTTGAAAATGACCGAC  
CAAGCGACGCCCAACCTGCCATCACGATGGCCCAATAAAATATCTTTATTTTCATTACA  
TCTGTGTGTTGGTTTTTTGTGTGAATCGATAGCGATAAGGATCCGCGTATGGTGCATCT  
CAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGC  
TGACGCGCCCTGACGGGCTTGTCTGCTCCCGCATCCGCTTACAGACAAGCTGTGACCGT  
CTCCGGGAGCTGCATGTGTGAGAGTTTTACCGTCTATCACCAGAACGCGCGAGACGAAA  
GGGCTCGTGATACGCCTATTTTTATAGGTTAATGTGATGATAATAATGGTTTTCTTAGAC  
GTCAGGTGGCACTTTTCGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAAT  
ACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTG  
AAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCCCCTTATCCCTTTTTTGCGGC  
ATTTTGCCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGA  
TCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGA  
GAGTTTTTCGCCCCGAAGAAGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGG  
CGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCT  
TCAGAAATGACTTGTTGAGTACTCACGAGTACAGAAAAGCATCTTACGGATGGCATGAC  
AGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACT  
TCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCAACATGGGGGATCA  
TGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCG  
TGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAAGTGGCGAACT  
ACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGG  
ACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGG  
TGAGCGTGGGTCTCGCGGTATCATTTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTAT  
CGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGC  
TGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATAT  
ACTTTAGATTGATTTAAAACTTCATTTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTT  
TGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCC  
CGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTT  
GCAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAAC  
TCTTTTTCCGAAGGTAACCTGGCTTACGAGAGCGCAGATACCAATACTGCTCTCTAGT  
GTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCT  
GCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGGTTGGA  
CTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGGTTCGTGCAC-

FIGURE 92C

ACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTG  
AGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGT  
CGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAACGCCTGGTATCTTTATAGTCC  
TGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG  
GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCC  
TTTTGCTCACATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGC  
CTTTGAGTGAGCTGATACCGCTCGCCGCGAGCCGAACGACCGAGCGCAGCGAGTCAGTGAG  
CGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCGCGCGTTGGCCGATTCA  
TTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGAAGCATTATCAGGGTTA  
TTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCC  
GCGCACATTTCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATT  
AACCTATAAAAATAGGCGTAGTACGAGGCCCTTCACTCATTAG

FIGURE 92D



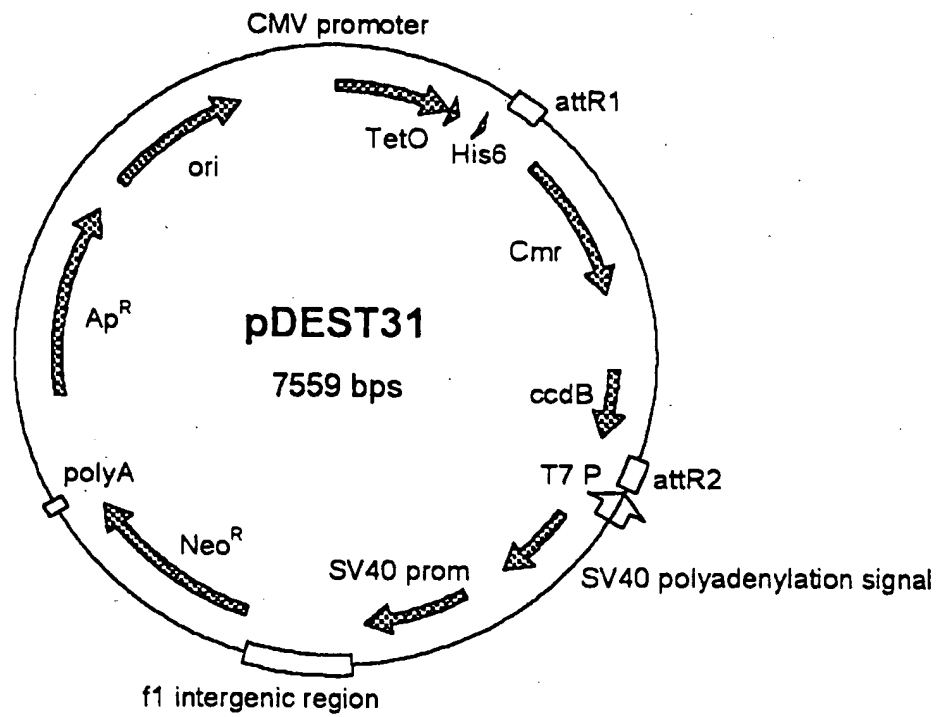


FIGURE 93A

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pDEST31 7559 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCC  
CGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGTATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC  
TCACGGGGATTTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTTGTTTTGGCACCAA  
AATCAACGGGACTTTTCCAAATGTCTGTAACAACTCCGCCCCATTGACGCAAATGGGCGGT  
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTTCGACGAGCTCGTTTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACC  
ATGGCGTACTACCATCACCATCACCATCACACCGGTGATATCCTCGAGCCCATCACAAGT  
TTGTACAAAAAAGCTGAACGAGAAACGTAATAATGATATAAATATCAATATATTAAATTAG  
ATTTTGCATAAAAAACAGACTACATAACTGTAAAACACAACATATCCAGTCACTATGG  
CGGCCGCTTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGA  
TTTTGAGTTAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAA  
TCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCAT  
TTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTTTCAGTGGATATTACGGCCTTTT  
TAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTACATTCTTGCCC  
GCCTGATGAATGCTCATCCGGAATTCGGTATGGCAATGAAAGACGGTGAGCTGGTGATAT  
GGGATAGTGTTCACCCCTTGTACACCGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGC  
TCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTGCAAGATGTGG  
CGTGTTACGGTGAAAACTGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCG  
TCTCAGCCAATCCCTGGGTGAGTTTTACCAGTTTTGATTTAAACGTGGCCAATATGGACA  
ACTTCTTCGCCCCCGTTTTTACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGA  
TGCCGCTGGCGATTTCAGGTTTCATCATGCCGTCTGTGATGGCTTTCATGTTCGGCAGAATGC  
TTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAACGCGTGGATCCG  
GCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAA  
TATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTAT  
TACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATC  
TCCGGTCTGGTAAGCACAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGG  
AAAGCGGAAAATCAGGAAGGGATGGCTGAGGTCCGCCGTTTTATTGAAATGAACGGCTCT  
TTTGCTGACGAGAACAGGGAAGTGGTGAATGTCAGTTTTAAGTTTTACACCTATAAAAGAGA  
GAGCCGTTATCGTCTGTTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGCGACG  
GATGGTGATCCCCCTGGCCAGTGCACGTCTGCTGTGATATAAAGTCTCCCGTGAACCTTA  
CCCCGGTGGTGATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGT  
GCCGGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAA  
AAACGCCATTAACCTGATGTTCTGGGGAATATAAATGTGAGGCTCCGTTATACACAGCCA  
GTCTGCAGGTGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTT  
TTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTCAG  
CTTTCTGTACAAAGTGGTGATGGGCGCGCTCTAGAGGGCCCAAGCTTACGCGTGCAT  
GCGACGTATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTCGT  
TTTACAACGTCGTGACTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTT  
CTGTGGTGTGACATAATTGGACAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATAT  
AAAATTTTTAAGTGTATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTT  
GCTTACTGAGTATGATTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTG  
TGATTTTTAGATTCACAGTCCCAAGGCTCATTTCAGGCCCTCAGTCTTCACAGTCTGTT  
CATGATCATAATCAGCCATACACATTTGTAGAGTTTTACTTGCTTTAAAAAACCTCCC  
ACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTTAT  
TGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCAAAATTTACAAATAAAGCATT  
TTTTTCACTGCATTCTAGTTGTGGTTTTGTCCAACTCATCAATGTATCTTATCATGTCTG  
GATCGATCCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCT  
GGCGTAATAGCGAAGAGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATG  
GCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA  
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTCCCTTCT  
TTCTCGCCACGTTTCGCCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT-

Figure 93B

TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTTCAC  
GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT  
TTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTT  
TTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAAC  
AAATATTTAACCGCAATTTTAACAAAATATTAACGTTTACAATTTTCGCCTGATGCGGTAT  
TTTCTCCTTACGCATCTGTGCGGTATTTACACCGCATACGCGGATCTGCGCAGCACCAT  
GGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACC  
AGCTGTGGAATGTGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAA  
GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCC  
CAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCC  
TAACTCCGCCCCATCCCGCCCCCTAACTCCGCCCAGTTCGCCCCATTCTCCGCCCCATGGCT  
GACTAATTTTTTTTATTTATGTCAGAGGCCGAGGCGCCTCGGCCCTCTGAGCTATTCCAGA  
AGTAGTGAGGAGGCTTTTTTGGAGGCCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACA  
CAACAGTCTCGAACTTAAGGCTAGAGCCACCATGATTGAACAAGATGGATTGCACGCAGG  
TTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGG  
CTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAA  
GACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCT  
GGCCACGACGGGCGTTCTTTCGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGA  
CTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCTCATCTCACCTTGCTCCTGC  
CGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTAC  
CTGCCCCATTGCAACCAAGCGAAACATCGCATCGAGCGAGCACGTAAGTTCGGATGGAAGC  
CGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGT  
GTTCCGCCAGGCTCAAGGCGCGCATGCCGACGGCGAGGATCTCGTCTGACCCATGGCGA  
TGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGG  
CCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGA  
AGAGCTTGGCGGCAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGA  
TTCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGAAGTCTGGGG  
TTGAAATGACCGACCAAGCGACGCCCCAAGTCCCATCACGATGGCCGCAATAAAATATC  
TTTATTTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGATAGCGATAAGGATCCG  
CGTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACA  
CCCCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAG  
ACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGTTTTTACCGTCATCACCGAA  
ACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAAT  
AATGGTTTTCTTAGACGTAGGTGGCACTTTTCGGGGAATGTGCGCGGAACCCCTATTTG  
TTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT  
GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCACATTTCCGTGTCGCCCTTAT  
TCCCTTTTTTTCGGGCATTTTGCCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGT  
AAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAG  
CGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTTCCAATGATGAGCACTTTTAA  
AGTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTG  
CCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCT  
TACGGATGGCATGACAGTAAGAGAATTATGCACTGCTGCCATAACCATGAGTGATAACAC  
TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCA  
CAACATGGGGGATCATGTAAGTGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT  
ACCAAACGACGAGCGTGACACCACGATGCTGTAGCAATGGCAACAACGTTGCGCAAACT  
ATTAAGTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGC  
GGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTTATTGCTGA  
TAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGG  
TAAGCCCTCCCGTATCGTAGTTATCTACACGCGGGGAGTCAGGCAACTATGGATGAACG  
AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAAGCATTGGTAACTGTGACACCA  
AGTTTACTCATATATACTTTAGATTGATTTAAACTTTCATTTTAAATTTAAAGGATCTA  
GGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCTGCCA  
CTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCG  
CGTAATCTGCTGCTTGCAACAAAAAACCACCGCTACCAGCGGTGGTTTGTTCGCCGA  
TCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAAA  
TACTGTCTTCTAGTGTAGCGGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCC  
TACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGT  
TCTTACCGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGCTGAAC-

FIGURE 93C

GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCT  
ACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC  
GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG  
GTATCTTTATAGTCCTGTCTGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATG  
CTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCT  
GGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA  
TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCGAGCCGAACGACCGAGCG  
CAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGC  
GCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGAAG  
CATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAA  
ACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCAT  
TATTATCATGACATTAACCTATAAAAAATAGGCGTAGTACGAGGCCCTTCACTCATTAG

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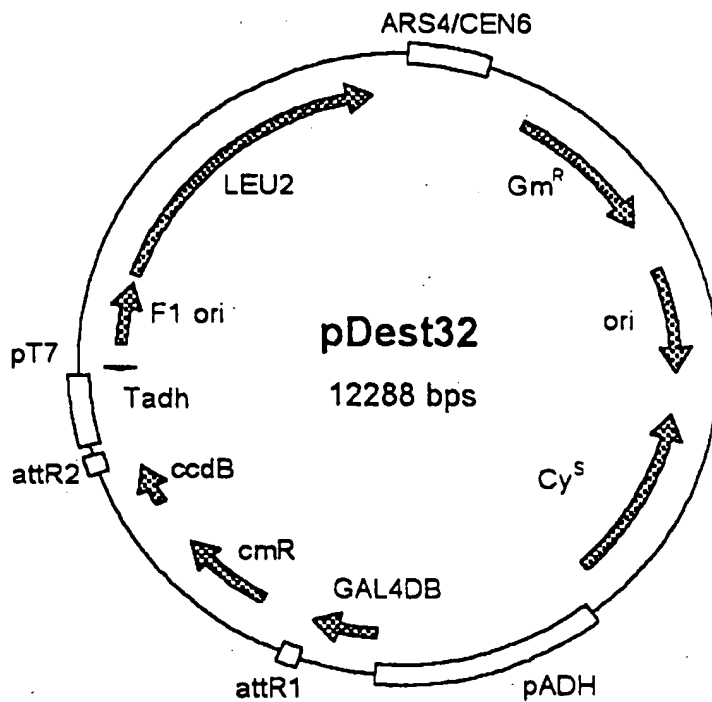


FIGURE 94A

pDEST32 12288 bp

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTT  
CTTAGGACGGATCGCTTGCCTGTAACCTACACGCGCCTCGTATCTTTAATGATGGAATA  
ATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTTGTATTTGGATTTTAGAAAGT  
AAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAATAAACAAGGTTTAAAAA  
ATTTCAACAAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATA  
GATATACATTTCGATTAACGATAAGTAAATGTAAATCACAGGATTTTCGTGTGTGGTCT  
TCTACACAGACAAGATGAAACAATTCCGCATTAATACCTGAGAGCAGGAAGAGCAAGATA  
AAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAAAACAAAACCT  
ATTTTTTCTTTAATTTCTTTTTTACTTTCTATTTTAAATTTATATATTTATATTAATAA  
ATTTAAATTATAATTATTTTTATAGCACGTGATGAAAAGGACCCAGGTGGCACTTTTCGG  
GGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAATACATTCAAATATGTATCCG  
CTCATGAGACAATAACCCCTGATAAATGCTTCAATAATCTGCAGTGCAGGGCCCGTGTCT  
TCAAATCTCTGATGTTACATTGCACAAGATAAAAAATATATCATCATGAACAATAAACT  
GTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTC  
TTGCTGGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGC  
TCGGTAGCCAACCACTAGAACTATAGCTAGAGTCTCGGGCGAACAAACGATGCTCGCCTT  
CCAGAAAAACCGAGGATGCGAACCACTTCATCCGGGGTCAACACCACCGCAAGCGCCGG  
ACGGCCGAGGTCTTCGATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGT  
AGAAGAACAGCAAGGCCGCAATGCCTGACGATGCGTGGAGACCGAAACCTTGCGCTCGT  
TCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTGCTGCCCAAGGTTGCCGGGTGACGCA  
CACCGTGGAACGGATGAAGGCACGAACCCAGTTGACATAAGCCTGTTTCGGTTTCGTAAAC  
TGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAACCTTGACCGAACGCAGCG  
GTGTAACGGCGCAGTGGCGGTTTTCATGGCTTGTTATGACTGTTTTTTGTACAGTCTA  
TGCCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTGATGTTTGATGTTATGGA  
GCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCACATGTAGGCTCGGCCCTAAACA  
AAGTTAGGTGGCTCAAGTATGGGCATATTGCGACATGTAGGCTCGGCCCTGACCAAGTC  
AAATCCATGCGGGCTGCTCTTGATCTTTTCGGTTCGTGAGTTCCGAGACGTAGCCACCTAC  
TCCCAACATCAGCCGACTCCGATTACCTCGGGAACCTTGCTCCGTAGTAAGACATTCATC  
GCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTTCTGCCC  
AGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTCGCAGTCTCCGGCGAGCAC  
CGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAAGCATGAGGCCAACCGCTT  
GGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCGCAGTGGCTCTCTAT  
ACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATCGACCCAAGTACCGCCACC  
TAACAATTTCGTTCAAGCCGAGATCGGCTTCCCGGCCATAAGGTTGTATTGATGTTGGAC  
GAGTCGGAATCGCAGACCGATACAGGATCTTGCCATCCTATGGAAGTGCCTCGGTGAGT  
TTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGA  
ATAAATTGCAGTTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGT  
TGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGNCATGACCAAAATCCCTT  
AACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT  
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAG  
CGGTGGTTTGTGTCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCA  
GCAGAGCGCAGATACCAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCA  
AGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACAGTGGCTGCTG  
CCAGTGGCGATAAGTCGTGCTTACCGGGTTGACTCAAGACGATAGTTACCGGATAAGG  
CGCAGCGGTCCGGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCT  
ACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGA  
GAAAGGCGGACAGGTATCCGTAAGCGGCAGGGTCGGAACAGGAGCGCACGAGGGAGC  
TTCCAGGGGGGAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTG  
AGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAACGCCAGCAACG  
CGGCCTTTTTACGGTTCTTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCGT  
TATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCC  
GCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATAC  
GCAAACCGCCTCTCCCCGCGGTTGGCCGATTCAATATGCAGCTGGCACGACGAGTTTC  
CCGACTGGAAAGCGGCGAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACTCATTAGG  
CACCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTGAGCGGAT  
AACAAATTTACACAGGAACAGCTATGACCATGATTACGCCAAGCTCGGAATTAACCTC-

FIGURE 94B

ACTAAAAGGGAACAAAAGCTGGTACCGATCCCGAGCTTTGCAAATTAAAGCCTTCGAGCGT  
CCCCAAAACCTTCTCAAGCAAGGTTTTCAGTATAATGTTACATGCGTACACGCGTCTGTAC  
AGAAAAAAGAAAAATTTGAAATATAAATAACGTTCTTAATACTAACATAACTATAAAA  
AAATAAATAGGGACCTAGACTTCAGGTTGTCTAACTCCTTCCTTTTCGGTTAGAGCGGAT  
GTGGGGGGAGGGCGTGAATGTAAGCGTGACATAACTAATTACATGATATCGACAAAGGAA  
AAGGGGCGCTGTTTACTCACAGGCTTTTTTCAAGTAGGTAATTAAGTCGTTTCTGTCTTTT  
TCCTTCTTCAACCCACCAAAGGCCATCTTGGTACTTTTTTTTTTTTTTTTTTTTTTTTTT  
TT  
TTTTTTTTCATAGAAATAATACAGAAGTAGATGTTGAATTAGATTAAACTGAAGATATAT  
AATTTATTGGAAAATACATAGAGCTTTTTGTTGATGCGCTTAAGCGATCAATTCAACAAC  
ACCACCAGCAGCTCTGATTTTTTCTTCAGCCAACCTGGAGACGAATCTAGCTTTGACGAT  
AACTGGAACATTTGGAATTTTACCCTTACCCAAGATCTTACCGTAACCGGCTGCCAAAGT  
GTCAATAACTGGAGCAGTTTCTTAGAAGCAGATTTCAAGTATTGGTCTCTCTTGTCTTC  
TGGGATCAATGTCACAAATTTGTCCAAGTTCAAGACTGGCTTCAGAAATGAGCTTGTTG  
CTTGTGGAAGTATCTCATACCAACCTTACCGAAATAACCTGGATGGTATTTATCCATGTT  
AATTTCTGTGGTGTGTTGACCACCGGCCATACCTCTACCACCGGGGTGCTTTCTGTGCTT  
ACCGATACGACCTTTACCGGCTGAGACGTGACCTCTGTGCTTTCTAGTCTTAGTGAATCT  
GGAAGGCATTCTTGATTAGTTGGATGATTGTTCTGGGATTTAATGCAAAAATCACTTAAG  
AAGGAAAATCAACGGAGAAAGCAAACGCCATCTTAAATATACGGGATACAGATGAAAGGG  
TTTGAACCTATCTGGAAAATAGCATTAAACAAGCGAAAACTGCGAGGAAAAATGTTTGC  
GTCTCTGCGGGCTATTACGCGCCAGAGGAAAAATAGGAAAAATAACAGGGCATTAGAAAA  
ATAATTTTGTGATTTTGGTAATGTGTGGGTCTGTTGTACAGATGTTACATTGGTTACAGTA  
CTCTTGTTTTTGCTGTGTTTTTCGATGAATCTCCAAAATGGTTGTTAGCACATGGAAGAG  
TCACCGATGCTAAGTTATCTCTATGTAAGCTACGTGGCGTGACTTTTGATGAAGCCGCAC  
AAGAGATACAGGATTGGCAACTGCAAATAGAATCTGGGGATCCCCCTCGAGATCCGGGA  
TCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATGAAGGCAAAAGACAAATA  
TAAGGGTCGAACGAAAAATAAAGTGAAAAGTGTGATATGATGTATTTGGCTTTGCGCGC  
CCGAAAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCTGTGGCGGACCCGCGCTC  
TTGCCGGCCCCGGCGATAACGCTGGGCGTGAGGCTGTGCCCGCGGAGTTTTTTGCGCCTG  
CATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGAAGCAATAAGAATGCCGG  
TTGGGGTTGCGATGATGACGACCACGACAACCTGGTGTGCTATTATTTAAGTTGCCGAAAGAA  
CCTGAGTGCTTTTGCAACATGAGTATACTAGAAGAATGAGCCAAGACTTGCGAGACGCGA  
GTTTGGCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAGGTGAGACGCGCATAACC  
GCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCAGTATAAATAGACAGGTA  
CATAACAACACTGGAAATGGTTGTCTGTTTGAAGTACGCTTTCAATTCAATTGGGTGTGCAC  
TTTATTATGTTTACAATATGGAAGGGAACCTTACACTTCTCCTATGCACATATATTAATTA  
AAGTCCAATGCTAGTAGAGAAGGGGGTAACACCCCTCCGCGCTCTTTTCCGATTTTTTT  
CTAAACCGTGGAATATTTCCGATATCCTTTTTGTTGTTTCCGGGTGTACAATATGGACTTC  
CTCTTTTCTGGCAACCAAACCCATACATCGGGATTCTATAATACCTTCGTTGGTCTCCC  
TAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATACCAGACAAGACATAATG  
GGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTGGTACATAACGAACATAAT  
ACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTCACTACCCTTTTTCCATT  
TGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTCTTTTTTTTTCTTTTCTC  
TCTCCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAAATGATGGAAGACACTAA  
AGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTGTTCCAGAGCTGATGAGG  
GGTATCTTCGAACACACGAACTTTTTCTTCTTCTTCACTCACGCACACTACTCTCTAATG  
AGCAACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAATAAAAAAAGTTTGCCGC  
TTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTGTTCCTCGTCATTGTTT  
TCGTTCCCTTTCTTCTTGTCTTTTTCTGACACAATATTTCAAGCTATACCAAGCATAC  
AATCAACTCCAAGCTTGAAGCAAGCCTCCTGAAAGATGAAGCTACTGTCTCTATCGAAC  
AAGCATGCGATATTTGCCGACTTAAAAAGCTCAAGTGCTCCAAAGAAAAACCGAAGTGCG  
CCAAGTGCTGAAGAACAACCTGGGAGTGTCGCTACTCTCCAAAACCAAAGGTCTCCGC  
TGACTAGGGCACATCTGACAGAAGTGAATCAAGGCTAGAAAGACTGGAACAGCTATTTT  
TACTGATTTTTCTCGAGAAGACCTTGACATGATTTTGAATGGAATCTTTACAGGATA  
TAAAGCATTTGTTAACAGGATTATTTGTACAAGATAATGTGAATAAAGATGCCGTACAG  
ATAGATTGGCTTCAGTGGAGACTGATATGCCCTTAACATTGAGACAGCATAGAATAAGTG  
CGACATCATCGGAAGAGAGTAGTAACAAAGGTCAAAGACAGTTGACTGTATCGTCCA  
GGTCAATCAAACAAGTTTGTACAAAAAGCTGAACGAGAAACGTAAATGATATAAATA-

TCAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAACTGTAAAAACACAAC  
ATATCCAGTCACTATGGCGGCCGCTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGA  
ATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGA  
TACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGG  
TTCCAACCTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTATCGAG  
ATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCACCGTTGA  
TATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCAATGTAC  
CTATAACCAGACCGTTTCAGCTGGATATTACGGCCCTTTTAAAGACCGTAAAGAAAAATAA  
GCACAAGTTTTATCCGGCCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGA  
ATTCCTGATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTTACCCTTGTTA  
CACCGTTTTTCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGA  
TTTCCGGCAGTTTCTACACATATATTTCGAAGATGTGGCGTGTTACGGTGAAAACCTGGC  
CTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAG  
TTTACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCGTTTTTCAC  
CATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTCA  
TCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGCTTAATGAATTACAACAGTACTG  
CGATGAGTGCCAGGGCGGGCGTAATCTAGAGGATCCGGCTTACTAAAAGCCAGATAACA  
GTATGCGTATTTGCGCGCTGATTTTTCGGGTATAAGAATATATACTGATATGTATACCCG  
AAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGAC  
AGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCA  
TGCAGAATGAAGCCCGTCGTCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGA  
TGGCTGAGGTGCGCCCGGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACT  
GGTGAATGCAAGTTTAAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTG  
GATGTACAGAGTGATATTATTGACACGCCCGGGCGACGGATGGTGATCCCCCTGGCCAGT  
GCACGTCTGCTGTGATATAAGTCTCCCGTGAACCTTTACCCGGTGGTGATATCGGGGAT  
GAAAGCTGGCGCATGATGACACCGGATATGGCCAGTGTCGGGTCTCCGTTATCGGGGAA  
GAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTT  
TGGGGAATATAAATGTGAGGCTCCCTTATACACAGCCAGTCTGCAGGTGACCATAGTGA  
CTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTATGCAAAATCTAATTTAA  
TATATTGATATTTATATCATTTTACGTTTCTCGTTTCAGCTTTCTTGTAACAAAGTGGTTG  
ATGGCCGCTAAGTAAGTAAGACGTCGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTGG  
AGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTCCGCTGTG  
TACCTTGCCAGAAATTTACGAAAAGATGGAAAGGGTCAAATCGTTGGTAGATACGTTGT  
TGACACTTCTAAATAAGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTAT  
AAAAAAAATAAGTGTATACAAATTTTAAAGTGAACCTTAGGTTTTTAAACGAAAATTTCTT  
GTTCTTGAGTAACTCTTTCCTGTAGGTGAGGTTGCTTTCTCAGGTATAGCATGAGGTGCG  
TCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCCATTT  
CACCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGATTTTTA  
TGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCCAATTCGCCCTA  
TAGTGAGTTCGTATTACAATTCAGTGGCCGTCGTTTTTACAACGTGCTGACTGGGAAAACCC  
TGGCGTTACCCAACCTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAG  
CGAAGAGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGAC  
GCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCT  
ACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCTTCTCGCCACG  
TTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGT  
GCTTTACGGCACCTCGACCCCAAAAACCTTGATTAGGGTGATGGTTACGTAAGTGGGCCA  
TCGCCCTGATAGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGA  
CTCTTGTTCCAAACTGGAACAACACTCAACCCCTATCTCGGTCTATTCTTTTGATTTATAA  
GGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAAC  
GCCAATTTTAAACAAAATATTAACGTTTACAATTTCTGATGCGGTATTTTCTCCTTACGC  
ATCTGTGCGGTATTTACACCGCATATCGACCGGTGAGGAGAACTTCTAGTATATCCAC  
ATACCTAATATATTGCTTATTAATAATGGAATCGGAACAATTACATCAAAATCCACAT  
TCTCTTCAAAATCAATTGCTCTGACTTCTTGTTCATGTGTGTTCAAAAACGTTTATATT  
TATAGGATAATTATACCTATTTCTCAACAAGTAATTGGTTGTTTGGCCGAGCGGTCTAA  
GGCGCCTGATTCAGAAATATCTTGACCGCAGTTAACTGTGGGAATACTCAGGTATCGTA  
AGATGCAAGAGTTTCAATCTCTTAGCAACCATTATTTTTTTCTCAACATAACGAGAACA  
CACAGGGGCGCTATCGCACAGAATCAAATTCGATGACTGGAAATTTTTTGTTAATTTTCAG  
AGGTGCGCTGACGCATATACCTTTTTCACTGAAAATTTGGGAGAAAAGGAAAGGTGAG-

FIGURE 94D



AGGCCGGAACCGGCTTTTCATATAGAATAGAGAAGCGTTCATGACTAAATGCTTGCAATCA  
CAATACTTGAAGTTGACAATATTATTTAAGGACCTATTGTTTTTCCAATAGGTGGTTAG  
CAATCGTCTTACTTTCTAACTTTTCTTACCTTTTACATTTTCAGCAATATATATATATATT  
TCAAGGATATACCATTCTAATGTCTGCCCCTATGTCTGCCCCTAAGAAGATCGTCCGTTTT  
GCCAGGTGACCACGTTGGTCAAGAAATCACAGCCGAAGCCATTAAAGGTTCTTAAAGCTAT  
TTCTGATGTTTCGTTCCAATGTCAAGTTTCGATTTTCGAAAAATCATTAAATTGGTGGTGCTGC  
TATCGATGCTACAGGTGTCCCACTTCCAGATGAGGCGCTGGAAGCCTCCAAGAAGGTTGA  
TGCCGTTTTGTTAGGTGCTGTGGGTGGTCTAAATGGGGTACCGGTAGTGTAGACCTGA  
ACAAGGTTTACTAAAAATCCGTAAAGAACTTCAATTGTACGCCAACTTAAGACCATGTAA  
CTTTCATCCGACTCTCTTTTAGACTTATCTCCAATCAAGCCACAATTTGCTAAAGGTAC  
TGACTTCGTTGTTGTGTCAGAGAATTAGTGGGAGGTATTTACTTTGGTAAGAGAAAGGAAGA  
CGATGGTGATGGTGTGCTTGGGATAGTGAACAATACACCGTTCCAGAAGTGCAAGAAT  
CACAAGAATGGCCGCTTTTCATGGCCCTACACATGAGCCACCATTGCCTATTTGGTCCTT  
GGATAAAGCTAATGTTTGGCCTCTTCAAGATTATGGAGAAAACTGTGGAGGAAACCAT  
CAAGAACGAATCCCTACATTGAAGGTTCAACATCAATTGATTGATTCTGCCGCCATGAT  
CCTAGTTAAGAACCCAACCCACCTAAATGGTATTATAATCACCAGCAACATGTTTGGTGA  
TATCATCTCCGATGAAGCCTCCGTTATCCCAGGTTCTTGGGTTTTGTTGCCATCTGCGTC  
CTTGGCCTCTTTGCCAGACAAGAACACCGCATTGTTGTTGTACGAACCATGCCACGGTTC  
TGCTCCAGATTTGCCAAAGAATAAGGTTGACCCTATCGCCACTATCTGTCTGCTGCAAT  
GATGTTGAAATTGTCATTGAACCTGCCTGAAGAAGGTAAGGCCATTGAAGATGCAGTTAA  
AAAGGTTTTGGATGCAGGTATCAGAACTGGTGATTTAGGTGGTTCCAACAGTACCACCGA  
AGTCGGTGATGCTGTGCGCCGAAGAAGTTAAGAAAATCCTTGCTTAAAAAGATTCTCTTTT  
TTTATGATATTTGTACATAAACTTTATAAATGAAATTCATAATAGAAACGACACGAAATT  
ACAAAATGGAATATGTTTCATAGGGTAGACGAACTATATACGCAATCTACATACATTTAT  
CAAGAAGGAGAAAAAGGAGGATAGTAAAGGAATACAGGTAAGCAAATTGATACTAATGGC  
TCAACGTGATAAGGAAAAAGAATTGCACTTTAACATTAATATTGACAAGGAGGAGGGCAC  
CACACAAAAAGTTAGGTGTAAACAGAAAATCATGAACTACGATTCTTAATTTGATATTGG  
AGGATTTTCTCTAAAAAATAAATAACAATAAAAAAACAATGACCTGACCAT  
TTGATGGAGTTTAAAGTCAATACCTTCTTGAACCATTTCCCATATGGTGAAAGTTCCCTC  
AAGAATTTTACTCTGTGAGAAACGGCCTTACGACGTAGTCGATATGGTGCACTCTCAGTA  
CAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACG  
CGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCG  
GGAGCTGCATGTGTGAGAGGTTTTACCGTCATCACCGAAACGCGCGA

FIGURE 94E

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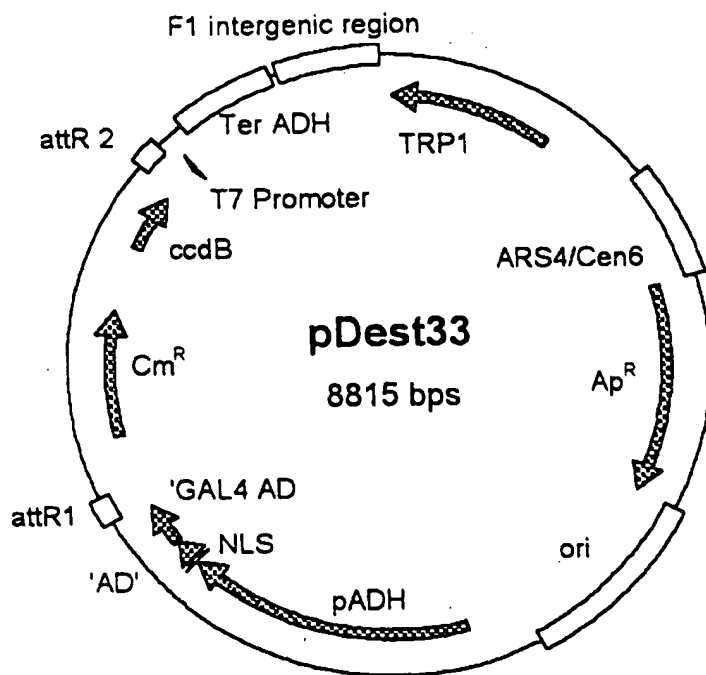


FIGURE 95A

pDEST33

8815 bp

GCCTTACGCATCTGTGCGGTATTTACACCCGAGGCAAGTGACAAACAATACTTAAATA  
AATACTACTCAGTAATAACCTATTTCTTAGCATTTTGGACGAAATTTGCTATTTTGTAG  
AGTCTTTTACACCATTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTA  
ATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGC  
TTTTCGGGGCTCTCTTGCCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTCAC  
CTGTCCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTG  
CACTGAGTAGTATGTTGCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGA  
GGAACCTCTTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGT  
AATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGT  
ATTTCCGGAGTGCCTGAACTATTTTATATGCTTTTACAAGACTTGAAATTTTCTTGCAA  
TAACCGGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCAT  
CGGAATCTAGAGCACATTCTGCGGCCCTCTGTGCTCTGCAAGCCGCAAACCTTTCACCAATG  
GACCAGAACTACCTGTGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAA  
TCACGTATACTCAGTGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTC  
TTTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAAGAAAGCTCCGGATCAAGATTGT  
ACGTAAGGTGACAAGCTATTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTC  
ATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCTATATATATA  
TATAGTAATGTCGTTTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA  
GCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGG  
CATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTAC  
CGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTA  
ATGTCATGATAATAATGGTTTTCTTAGGACGGATCGCTTGCCTGTAACCTACACGCGCCTC  
GTATCTTTTAATGATGGAATAATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTT  
TGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAA  
AAATAAACAAAGTTTAAAAAATTTCAACAAAAACGCTACTTTACATATATATTTATTAG  
ACAAGAAAAGCAGATTAAATAGATATACATTGATTAACGATAAGTAAAATGTAAATCA  
CAGGATTTTTCGTGTGTGGTCTTCTACACAGACAAGATGAAACAATTCGGCATTAATACCT  
GAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTA  
CATCTTTCGGAAAAACAAAACCTATTTTTTCTTTAATTTCTTTTTTACTTTCTATTTTTAA  
TTTATATATTTATATTAATAAATTTAAATTATAATTATTTTTATAGCACGTGATGAAAAG  
GACCCAGGTGGCACTTTTCGGGGAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAA  
ATACATCAAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATAT  
TGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATCCCTTTTTTGCG  
GCATTTTGCCTTCTGTTTTTGGCTACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAA  
GATCAGTTGGGTGCACGAGTGGGTTACATCGAAGTGGATCTCAACAGCGGTAAGATCCTT  
GAGAGTTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGT  
GGCGCGGTATTATCCCGTATTGACGCGGGCAAGAGCAACTCGGTGCGCCGATACACTAT  
TCTCAGAATGACTTGGTTGAGTACTACCAAGTCACAGAAAAGCATCTTACGGATGGCATG  
ACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAACACTGCGGCCAACTTA  
CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGAT  
CATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG  
CGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAAGTGGCGAA  
CTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA  
GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCC  
GGTGAGCGTGGGTCTCGCGGTATCATTCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT  
ATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATC  
GCTGAGATAGGTGCCTCACTGATTAAGCATTTGGTAACTGTCAGACCAAGTTTACTCATAT  
ATACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAGGATCTAGGTGAAGATCCTT  
TTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGAC  
CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGC  
TTGCAAAACAAAAAACCCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCA  
ACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCTTCTA  
GTGTAGCCGTATAGGCCACCACTTCAAGAATCTGTAGCACCGCCCTACATACCTCGCT  
CTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG  
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGGCTGAACGGGGGGTTCTGTGC  
ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCAT-

FIGURE 95B

TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG  
GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGT  
CCTGTGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGG  
CCGAGCCTATGGAACCGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGG  
CCTTTTGCTCACATGTTCTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACC  
GCCTTTGAGTGAGCTGATACCGCTCGCCGACGCGAACCGCCTCTCCCCGCGCTTGGCCGATT  
AGCGAGGAAGCGGAAGAGCGCCCAATACGCAACCGCCTCTCCCCGCGCTTGGCCGATT  
CATTAAATGCAGCTGGCAGACAGGTTTCCCGACTGGAAGCGGGCAGTGAGCGCAACGCA  
ATTAATGTGAGTTACCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCT  
CCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCAT  
GATTACGCCAAGCTCGGAATTAACCCCTCACTAAAGGGAAACAAAGCTGGGTACCGGGCCC  
CCCCTCGAGATCCGGGATCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATG  
AAGGCAAAAGACAAATATAAGGGTCTGAACGAAAAAATAAAGTGAAAAGTGTGATATGATG  
TATTTGGCTTTGCGGCGCCGAAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCT  
GTGGCGGACCCGCGCTCTTGCCGGCCCGCGATAACGCTGGGCGTGAGGCTGTGCCCGGC  
GGAGTTTTTTGCGCCTGCATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGA  
AGCAATAAGAATGCCGTTGGGGTTGCGATGATGACGACCACGACAACCTGGTGTCTATTAT  
TTAAGTTGCCGAAAGAACCTGAGTGCATTTGCAACATGAGTATACTAGAAGAATGAGCCA  
AGACTTGCGAGACGCGAGTTTGCCGGTGGTGCGAACAAATAGAGCGACCATGACCTTGAAG  
GTGAGACGCGCATAACCGCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCA  
GTATAAATAGACAGGTACATAACAACCTGGAAATGGTTGTCTGTTGAGTACGCTTTCAA  
TTCATTTGGGTGTGCACTTTATTATGTTACAATATGGAAGGGAACTTTACACTTCTCCTA  
TGCACATATATTAATTAAAGTCCAATGCTAGTAGAGAAGGGGGGTAACACCCCTCCGCGC  
TCTTTTCCGATTTTTTTCTAAACCGTGGAATATTTTCGGATATCCTTTTGTGTTTCCGGG  
TGTACAATATGGACTTCTCTTTTCTGGCAACCAACCCATACATCGGGATTCTCTATAAT  
ACCTTCGTTGGTCTCCCTAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATA  
CCAGACAAGACATAATGGGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTG  
GTACATAACGAATAACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTC  
ACTACCTTTTTCATTTTGGCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTC  
TTTTTTTTTCTTTTCTCTCTCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAAA  
ATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTG  
TTCCAGAGCTGATGAGGGGTATCTTCGAACACACGAAACTTTTTCTTCTTTCATTACG  
CACACTACTCTCTAATGAGCAACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAA  
TAAAAAAAGTTTGCCGCTTGTCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTG  
TTTCTCGTCATTGTTCTCGTTCCCTTTCTTCTTGTCTTTTTTCTGCACAATATTTCA  
AGCTATACCAAGCATACAATCAACTCCAAGCTTATGCCCAAGAAGAAGCGGAAGGTCTCG  
AGCGGCGCCAATTTTAATCAAAGTGGAATATTGCTGATAGCTCATTGTCTTCACTTTC  
ACTAACAGTAGCAACGGTCCGAACCTCATAACAACTCAAACAAATTCTCAAGCGCTTTC  
CAACCAATTGCCTCCTCTAACGTTTCATGATACTTCATGAATAATGAAATCACGGCTAGT  
AAAATTGATGATGGTAATAATTCAAACCACTGTCACCTGGTTGGACGGACCAAACTGCG  
TATAACGCGTTTGGAACTCACTACAGGGATGTTTAATACCACTACAATGGATGATGATAT  
AACTATCTATTGATGATGAAGATACCCCAACCAACCAAAAAAGAGGGTGGGTGGAAT  
CAAACAAGTTTGTACAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATA  
TTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAACACAACATATCCAG  
TCACTATGGCGGCCGCTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGAATAAATAC  
CTGTGACGGAAGATCACTTCGAGAAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGA  
AGCCCTGGGCCAACTTTTGGCGAAAAATGAGACGTTGATCGGCACGTAAGAGGTTCCAACT  
TTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGTGTTATCGAGATTTTCAG  
GAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCACCGTTGATATATCCC  
AATGGCATCGTAAAGAACATTTTGGAGGCATTTTCAGTCAGTTGCTCAATGTACCTATAACC  
AGACCGTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAAAAATAAGCACAGT  
TTTATCCGGCCTTTTATTCATTTCTTGCCCGCCTGATGAATGCTCATCCGGAATTCGTA  
TGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTTACCCTTGTACACCGTTT  
TCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGC  
AGTTTCTACACATATATTCGCAAGATGTGGCGTGTACGGTGAAAACTGGCCTATTTCC  
CTAAAGGGTTTATTGAGAATATGTTTTTCTGCTCAGCCAATCCCTGGGTGAGTTTACCA  
GTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGTTTTTACCATGGGCA  
AATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTACAGGTTTCATCATGCCG-

FIGURE 95C

TCTGTGATGGCTTCCATGTCCGCGAGAATGCTTAATGAATTACAACAGTACTGCGATGAGT  
GGCAGGGCGGGGCGTAATCTAGAGGATCCGGCTTACTAAAAGCCAGATAACAGTATGCGT  
ATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTATACCCGAAGTATGT  
CAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGCTATCA  
GTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGCAGAAT  
GAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGATGGCTGAG  
GTCGCCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGTGAAAT  
GCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACA  
GAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGACAGTCT  
GCTGTGAGATAAAGTCTCCCGTGAACCTTTACCCGGTGGTGATATCGGGGATGAAAGCTG  
GCGCATGATGACCACCGATATGGCCAGTGTGCGGGTCTCCGTTATCGGGGAAGAAGTGCG  
TGATCTCAGCCACCGGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAAAT  
ATAAATGTCAGGCTCCGTTATACACAGCCAGTCTGCAGGTGACCATAGTGACTGGATAT  
GTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGA  
TATTTATATCATTTTACGTTTCTCGTTTCTGTTTCTGTACAAAGTGGTTTGTATGGCCGC  
TAAGTAAGTAAGACGTCGAGCTCCCTATAGTGAGTCGTATTACACTGGCCGTCGTTTTAC  
AACGTCGTGACTGGGAAAACACCGGTGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTG  
GAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTGCGCTTGT  
CTACCTTGCCAGAAAATTTACGAAAAGATGGAAGGGTCAAATCGTTGGTAGATACGTTG  
TTGACACTTCTAAATAAGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTA  
TAAAAAAATAAGTGTATACAAATTTTAAAGTGAAGTCTTAGGTTTTAAAACGAAAATTTCT  
TGTTCTTGAGTAAGTCTTTTCTGTAGGTGAGTTGCTTTCTCAGGTATAGCATGAGGTG  
CTCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCCATT  
TCACCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGTATTTT  
ATGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCGCATCAGGCGA  
AATTGTAAACGTTAATATTTTGTAAATTCGCGTTAAATATTTGTTAAATCAGCTCATT  
TTTTAACCAATAGGCCGAAATCGGCCAAAATCCCTTATAAATCAAAAGAATAGACCGAGAT  
AGGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAA  
CGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCCTA  
ATCAAGTTTTTTGGGGTGGAGGTGCGGTAAAGCACTAAATCGGAACCCCTAAAGGGAGCCC  
CCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGAAGAAAGC  
GAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCAACCAC  
ACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTGCGCCATTCACTGCA

FIGURE 95D

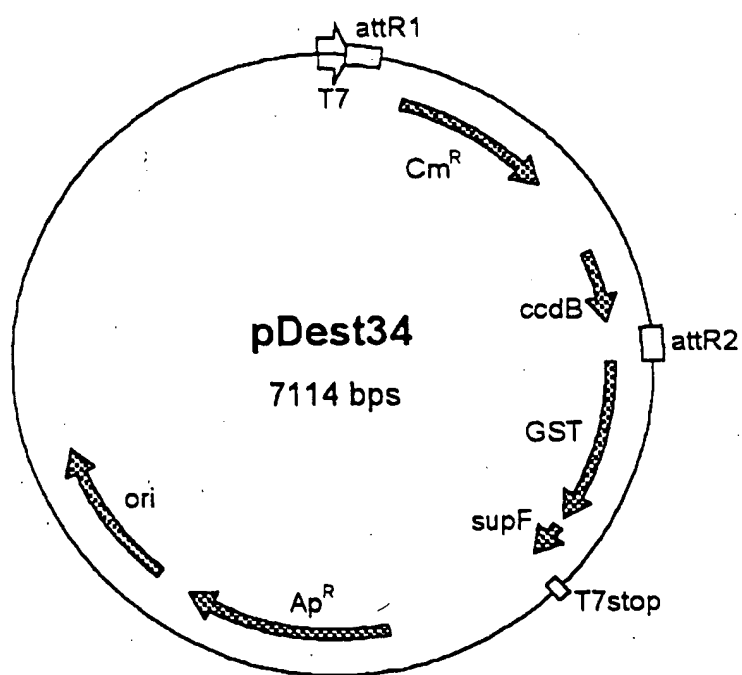


FIGURE 96A

## pDEST34 7114 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
195..71	attR1
304..963	CmR
1305..1610	ccdB
1651..1775	attR2
1780..2472	GST
2675..2720	T7stop
3334..4194	ampR
4343..4982	ori

ATCGAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTC  
 CCTCTAGATCACAAGTTTGTACAAAAAGCTGAACGAGAAACGTAAAATGATATAAATAT  
 CAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAAACACAACA  
 TATCCAGTCACTATGGCGGCCCGCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGC  
 TCGTATAATGTGTGGATTTTGTAGTTAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCT  
 AAAATGGAGAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAA  
 GAACATTTTGAGGCATTTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTTCAGCTG  
 GATATTACGGCCTTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTT  
 ATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTCGGTATGGCAATGAAAGAC  
 GGTGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAAC  
 GAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATA  
 TATTTCGCAAGATGTGGCGTGTTACCGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATT  
 GAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTGTATTTAAAC  
 GTGGCCAATATGGACAACCTTCTTCGCCCCCGTTTTTCACCATGGGCAAATATTATACGCAA  
 GGCGACAAGGTGCTGATGCGCGTGGCGATTTCAGGTTTCATCATGCCGTCTGTGATGGCTTC  
 CATGTCCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCG  
 TAAACCGGTGGATCCGGCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGAT  
 TTTTGCGGTATAAGAATATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTG  
 CTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCAT  
 ATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGCAGAATGAAGCCCGTCTGCT  
 GCGTGCCGAACGCTGGAAAGCGGAAATCAGGAAGGGATGGCTGAGGTGCGCCCGTTTTAT  
 TGAAATGAACGGCTCTTTTGTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAGGTTT  
 ACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTG  
 ACACGCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGCACGTCTGCTGTCAGATAAAG  
 TCTCCCGTGAACCTTTACCCGTTGGTGATATCGGGGATGAAAGCTGGCGCATGATGACCA  
 CCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACC  
 GCGAAAATGACATCAAAAACGCCATTAACTGATGTTCTGGGGAATATAAATGTCAAGCT  
 CCCTTATACACAGCCAGTCTGCAGGTGCACCATAGTGAAGTGGATATGTTGTGTTTTACAG  
 TATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTT  
 TACGTTTCTCGTTTCAGCTTTCTGTACAAAGTGGTGATTATGTCCCCTATACTAGGTTAT  
 TGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGAATATCTTGAAGAAAAA  
 TATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAA  
 TTGGGTTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACAG  
 TCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAA  
 GAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTTGGATATTAGATACGGTGTTTCG  
 AGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCT  
 GAAATGCTGAAAATGTTTCAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT  
 GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCA  
 ATGTGCCTGGATGCGTTCCAAAATTAGTTTGTGTTTTAAAAACGTATTGAAGCTATCCCA  
 CAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAA  
 GCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGGTTCCGCGTCCATGGGGA  
 TCCCGTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCGCTT  
 CCCGATAAGGGAGCAGGCCAGTAAAGCATTACCCGTGGTGGGTTCCCGAGCGGCCAA  
 GGGAGCAGACTCTAAATCTGCCGTATCGACTTCGAAGGTTTGAATCCTTCCCCCACCAC  
 CATCACTTTCAAAGTGAATTCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAA-

FIGURE 96B

ACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATATCCACAGGACGG  
GTGTGGTCGCCATGATCGCGTAGTCGATAGTGGCTCCAAGTAGCGAAGCGAGCAGGACTG  
GGCGGCGGCCAAAGCGGTCCGACAGTGCTCCGAGAACGGGTGCGCATAGAAATTGCATCA  
ACGCATATAGCGCTAGCAGCACGCCATAGTGACTGGCGATGCTGTGCGGAATGGACGATAT  
CCCGCAAGAGGCCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGA  
CGGTGCCGAGGATGACGATGAGCGCATTGTTAGATTTCATACACGGTGCCTGACTGCGTT  
AGCAATTTAACTGTGATAAACTACCGCATTAAAGCTTATCGATGATAAGCTGTCAAACAT  
GAGAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATG  
ATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCT  
ATTTGTTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGA  
TAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGC  
CTTATTCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCAGCCAGAAACGCTGGTG  
AAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTC  
AACGCGGTAAAGATCCTTGAGAGTTTTGCGCCCCGAAGAACGTTTTCCAATGATGAGCACT  
TTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTGACGCCGGGCAAGAGCAACTC  
GGTCCGCCGATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAG  
CATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGAT  
AACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCCTTTT  
TTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA  
GCCATACCAAACGACGAGCGTGACACCACGATGCCCTGCAGCAATGGCAACAACGTTGCGC  
AACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATG  
GAGGCGGATAAAGTTGCGAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATT  
GCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCA  
GATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGAT  
GAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCA  
GACCAAGTTTACTCATATATACTTTAGATTGATTTAAACTTTCATTTTTAATTTAAAGG  
ATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCG  
TTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT  
CTGCGCGTAATCTGCTGCTTGCAAACAAAAAACACCGCTACCAGCGGTGGTTTTGTTG  
CCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATA  
CCAAATACTGTCTTTCTAGTGATAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCA  
CCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAG  
TCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGC  
TGAACGGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAATGAGA  
TACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGG  
TATCCGGTAAGCGGCGGGTCCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAC  
GCCTGGTATCTTTATAGTCCTGTGCGGTTTTGCCACCTCTGACTTGAGCGTCGATTTTTG  
TGATGCTCGTCAGGGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGCCTTTTTACGG  
TTCTGCGCCTTTTTGCTGGCCTTTTTGCTCACATGTTCTTTCCTGCGTTATCCCTGATTCT  
GTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACC  
GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTCTCCTT  
ACGCATCTGTGCGGTATTTACACCGCATATATGGTGCACTCTCAGTACAATCTGCTCTG  
ATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTGATGGCTGC  
GCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATC  
CGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTACCGTC  
ATCACCGAAACGCGCGAGGCGAGCTGCGGTAAAGCTCATCAGCGTGGTTCGTGAAGCGATT  
ACAGATGTCTGCCTGTTTCATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGT  
CTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGTTTTTTCTGTTTGGTCACTGATGC  
CTCCGTGTAAGGGGATTTCTGTTTCATGGGGGTAATGATACCGATGAAACGAGAGAGGAT  
GCTCAGGATACGGGTACTGATGATGAACATGCCCGTTACTGGAACGTTGTGAGGGTAA  
ACAACTGGCGGTATGGATGCGGCGGGACAGAGAAAAATCACTCAGGGTCAATGCCAGCG  
CTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCATCCTGCGATGCAGAT  
CCGGAACATAATGGTGAGGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAA  
ACCGAAGACCATTCATGTTGTTGCTCAGGTCCGAGACGTTTTGCGAGCAGCAGTCGCTTCA  
CGTTCGCTCGCGTATCGGTGATTCACTCTGCTAACAGTAAGGCAACCCCGCCAGCCTAG  
CCGGGTCTCAACGACAGGAGCAGCATGTCGCGACCCGTTGGCCAGGACCCCAACGCTCC  
CGAGATGCGCCCGTGGCGCTGCTGAGATGGCGGACGCGATGGATATGTTCTGCCAAGG  
GTTGGTTTTGCGCATTACAGTTCTCCGCAAGAATTGATTGGCTCCAATTCTTGGAGTGGT-

FIGURE 96C



GAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTTCAGGTCGAGGTGGCCCCGGCTCCATGCA  
CCGCGACGCAACGCGGGGAGGCAGACAAGGTATAGGGCGGCGCCTACAATCCATGCCAAC  
CCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAGCGGTCCAGTGATC  
GAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTTCGTCTCT  
ACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAAGA  
ATCATAATGGGGAAGGCCATCCAGCCTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGC  
GCGTCGGCCGCCATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGA  
CCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCG  
ATCATCGTCGCGCTCCAGCGAAAGCGGTCTCTCGCCGAAAATGACCCAGAGCGCTGCCGGC  
ACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCGACGATAGTCATG  
CCCCGCGCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTTCGATCG  
ACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTT  
GAGCACCGCCGCCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGC  
CACGGGGCCTGCCACCATACCACGCCGAAACAAGCGCTCATGAGCCCAGAGTGGCGAGC  
CCGATCTTCCCCATCGGTGATGTGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCC  
GGTGATGCCGGCCACGATGCGTCCGGCGTAGAGG

FIGURE 9(a)

230/p40

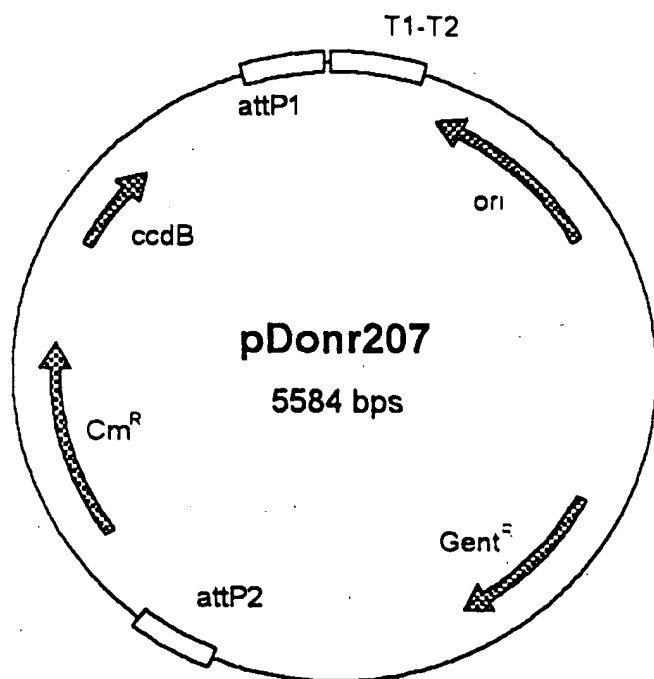


FIGURE 97A

pDONR207

5584 bp

CGGAGAGTAGGGAAGTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGGAAGACTGGGC  
CTTTTCGTTTTATCTGTTGTTTGTTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGG  
AGCGGATTTGAACGTTGTGAAGCAACGGCCCCGAGGGTGGCGGGCAGGACGCCCCGCATA  
AACTGCCAGGCATCAAACCTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGTTTCT  
ACAAACTCTTCCTGGCTAGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGA  
AAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTG  
GCGTTTTTCCATAGGCTCCGCCCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAG  
AGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTC  
GTGCGCTCTCCTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCG  
GGAAGCGTGGCGCTTTCTCATAGCTCAGCTCAGCTGTAGGTATCTCAGTTCCGGTGTAGTGGATT  
CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCC  
GGTAACCTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC  
ACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGG  
TGGCCTAACTACGGCTACACTAGAAGGACAGTATTGGTATCTGCGCTCTGCTGAAGCCA  
GTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGC  
GGTGGTTTTTTTTGTTTGCAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGAT  
CCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAACTCAGTTAAGGATTT  
TTGGTTCATGAGCTTGCGCCGTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACC  
AATTAACCAATTCTGATTAGAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCA  
TATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAACT  
CACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTC  
CAACATCAATACAACCTATTAGTAGCCAACCACTAGAACTATAGCTAGAGTCCTGGGCGA  
ACAAACGATGCTCGCCTTCAGAAAAACCGAGGATGCGAACCCTTCATCCGGGGTCAGCA  
CCACCGGCAAGCGCCGACGGCCGAGGTCTCCGATCTCCTGAAGCCAGGGCAGATCCG  
TGCACAGCACCTTGCCGTAGAAGAACAGCAAGGCCCAATGCCTGACGATGCGTGGAGA  
CCGAAACCTTGCGCTCGTTGCCAGCCAGGACAGAAATGCCTCGACTTCGCTGCTGCCCCA  
AGGTTGCCGGGTGACGCACACCGTGGAACCGGATGAAGGCACGAACCCAGTTGACATAAG  
CCTGTTCCGGTTCGTAAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAA  
CCTTGACCGAACCGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGTTATGACT  
GTTTTTTTGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGT  
GATGTTTGATGTTATGGAGCAGCAACGATGTTACGCGCAGCAACGATGTTACGCGCAG  
CTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCTGTAGTTC  
GGAGACGTAGCCACCTACTCCAACATCAGCCGGACTCCGATTACCTCGGGAACCTTGCTC  
CGTAGTAAGACATTTCATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTGTTGGCGCTCTC  
GCGGCTTACGTTCTGCCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTC  
GCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAAG  
CATGAGGCCAACCGCGCTTGGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGAT  
CCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATC  
GACCCAAGTACCGCCACCTAACAAATTCGTTCAAGCCGAGATCGGCTTCCCGGCCTAATTT  
CCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGG  
TGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACG  
CTCGTCATCAAATCACTCGCATCAACCAACCGTTATTCATTCTGTGATTGCGCCTGAGC  
GAGACGAAATACGCGATCGCTGTTAAAGGACAATTACAAACAGGAATCGAATGCAACCG  
GCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTCTAA  
TACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGT  
ACGGATAAAATGCTTGATGGTCCGGAAGAGGCATAAATTCGTCAGCCAGTTTAGTCTGAC  
CATCTCATCTGTAAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGG  
CGCATCGGGCTTCCCATACAAGCGATAGATTGTGCGACCTGATTGCCCGACATTATCGCG  
AGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGT  
TTCCCGTTGAATATGGCTCATAACCCCCCTGTATTACTGTTTATGTAAGCAGACAGTTT  
TATTGTTTCATGATGATATATTTTTATCTGTGCAATGTAACATCAGAGATTTTGAGACAC  
GGGCCAGAGCTGCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGACCTGTT  
CGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTG  
AACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAAC  
AGACTACATAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATG-

FIGURE 97B

GTATTAGTGACCTGTAGTCGACTAAGTTGGCAGCATCACCCGACGCACCTTTGCGCCGAAT  
AAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATA  
CCGGGAAGCCCTGGGCCAACTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTT  
CAACTTTTACCATAATGAAATAAGATCACTACCGGGCGTATTTTGTAGTTATCGAGATT  
TTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCACCGTTGATAT  
ATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCAATGTACCTA  
TAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTAAAGACCGTAAAGAAAAATAAGCA  
CAAGTTTTATCCGGCCTTTATTACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATT  
CCGTATGGCAATGAAAGACGGTGAGCTGGTATATGGGATAGTGTTTACCCTTGTTACAC  
CGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATT  
CCGGCAGTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTA  
TTTCCCTAAAGGGTTTTATTGAGAAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTT  
CACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTCTTCGCCCCCGTTTTTACCAT  
GGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTTCATCA  
TGCCGCTCTGTGATGGCTTCCATGTTCGGCAGAATGCTTAATGAATTACAACAGTACTGCCA  
TGAGTGGCAGGGCGGGGCGTAATCGCGTGGATCCGGCTTACTAAAAGCCAGATAACAGTA  
TGCGTATTTGCGCGCTGATTTTGGCGGTATAAGAATATATACTGATATGTATACCCGAAG  
TATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGC  
TATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGCTCTGGTAAGCACAACCATGC  
AGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAAGCGGAAAATCAGGAAGGGATGG  
CTGAGGTGCGCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGT  
GAAATGCAGTTTAAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGAT  
GTACAGAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGCA  
CGTCTGCTGTGAGATAAAGTCTCCCGTGAACCTTTACCCGGTGGTGATATCGGGGATGAA  
AGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGCTCTCCGTTATCGGGGAAGAA  
GTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGG  
GGAATATAAATGTGAGGCTCCCTTATACACAGCCAGTCTGCAGGTGATACAGTAGAAAT  
TACAGAACTTTTATCAGTTTTAGTAAGTATAGAGGCTGAAAATCCAGATGAAGCCGAACG  
ACTTGTAAGAGAAAAGTATAAGAGTTGTGAAATTGTTCTTGATGCAGATGATTTTCAGGA  
CTATGACACTAGCGTATATGAATAGGTAGATGTTTTTATTTTGTACACAAAAAAGAGGC  
TCGCACCTCTTTTTCTTATTTCTTTTTATGATTTAATACGGCATTGAGGACAAATAGCGAG  
TAGGCTGGATACGACGATTCCGTTTGAGAAGAACATTTGGAAGGCTGTCCGTCGACTAAG  
TTGGCAGCATCACCCGAAGAACATTTGGAAGGCTGTCCGTCGACTACAGGTCACTAATAC  
CATCTAAGTAGTTGATTCATAGTACTGGATATGTTGTGTTTTACAGTATTATGTAGTCT  
GTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTT  
CAGCTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACG  
AACAGGTCACTATCAGTCAAATAAAATCATTATTTGGGGCCCGAGATCCATGCTAGCGT  
TAAC

FIGURE 97C

pMAB85

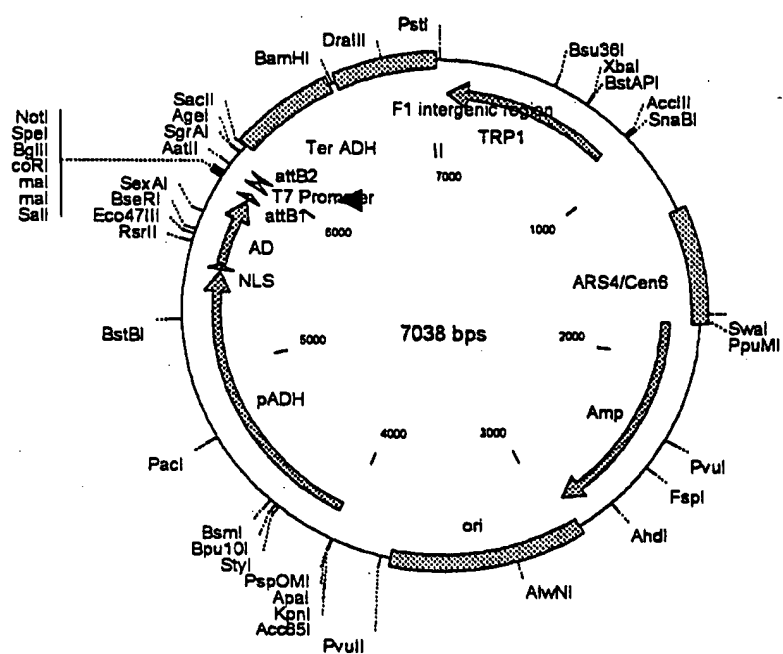


FIGURE 98A

pMAB85

7038 bp

GCCTTACGCATCTGTGCGGTATTTTCACACCGCAGGCAAGTGCACAAACAATACTTAAATA  
AATACTACTCAGTAATAACCTATTTCTTAGCATTTTGTGACGAAATTTGCTATTTTGTAG  
AGTCTTTTACACCATTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTA  
ATCTAAGCGCATACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGC  
TTTCGGGGCTCTCTTGCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTCAC  
CTGTCCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTG  
CACTGAGTAGTATGTTGCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGA  
GGAACCTCTTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGT  
AATCATTGACCAGAGCTAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGT  
ATTTTCGGAGTGCCTGAACTATTTTATATGCTTTTACAAGACTTGAAATTTTCTTGCAA  
TAACCGGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCAT  
CGGAATCTAGAGCACATTCTGCGGCCCTCTGTGCTCTGCAAGCCGCAAACTTTCACCAATG  
GACCAGAACTACCTGTGAAATTAATAACAGACATACTCCAAGCTGCCTTGTGTGCTTAA  
TCACGTATACTCAGTGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTC  
TTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAGAAAGCTCCGGATCAAGATTGT  
ACGTAAGGTGACAGCTATTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTC  
ATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATA  
TATAGTAATGTGTTTTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA  
GCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGG  
CATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTAC  
CGTCATCACCGAAACGCGCGAGACGAAAGGGCCCTCGTGATACGCCCTATTTTTATAGGTTA  
ATGTTCATGATAATAATGTTTTCTTAGGACGGATCGTTGCTGTAACCTTACACGCGCCTC  
GTATCTTTTAAATGATGGAATAATTGGGAATTTACTCTGTGTTTATTTATTTTATGTTT  
TGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAA  
AAATAAACAAAGGTTTTAAAAATTTCAACAAAAGCGTACTTTACATATATATTTATTAG  
ACAAGAAAAGCAGATTAAATAGATATACATTGATTAACGATAAGTAAATGTAAATCA  
CAGGATTTTCGTGTGTGGTCTTCTACACAGACAAGATGAAACAATTCGGCATTAAATACCT  
GAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTA  
CATCTTCGGAACAAAAAACTATTTTTCTTTAATTTCTTTTTTACTTTCTATTTTTAA  
TTTATATATTTTATATTTAAAAATTTAAATTATAATTATTTTATAGCACGTGATGAAAAG  
GACCCAGGTGGCACTTTTCGGGGAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAA  
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCGTGATAAATGCTTCAATAATAT  
TGAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATCCCTTTTTTGCG  
GCATTTTGCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA  
GATCAGTTGGGTGCACGAGTGGGTACATCGAAGTGGATCTCAACAGCGGTAAGATCCTT  
GAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGT  
GGCGCGGTATTATCCCGTATTGACGCGGGCAAGAGCAACTCGGTGCGCGCATACACTAT  
TCTCAGAATGACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCTTACGGATGGCATG  
ACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTA  
CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGAT  
CATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG  
CGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACCTGGCGAA  
CTACTTACTCTAGCTTCCCGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA  
GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGTGTATTGCTGATAAATCTGGAGCC  
GGTGAGCGTGGGTCTCGCGGTATCATTCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT  
ATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATC  
GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACCTGTGACACCAAGTTTACTCATAT  
ATACTTTAGATTGATTTAAACTTCATTTTTAATTTAAAGGATCTAGGTGAAGATCCTT  
TTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGAC  
CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGC  
TTGCAAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCA  
ACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCTTCTA  
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCT  
CTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG  
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGC-

FIGURE 98B

ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCAT  
TGAGAAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG  
GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGT  
CCTGTCGGGTTTTGCCACCTCTGACTTGAGCGTCGATTTTTTGTGATGCTCGTCAGGGGGG  
CCGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCTTGGCCTTTTGCTGG  
CCTTTTGCTCACATGTTCTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACC  
GCCTTTGAGTGAGCTGATACCGCTCGCCGACCCGAACGACCGAGCGCAGTCAGTG  
AGCGAGGAAGCGGAAGAGCGCCCAATACGCAAAACCGCCTCTCCCCGCGCGTTGGCCGATT  
CATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA  
ATTAATGTGAGTTACCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCT  
CCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAAACAGCTATGACCAT  
GATTACGCCAAGCTCGGAATTAACCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCC  
CCCCTCGAGATCCGGGATCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATG  
AAGGCAAAAGACAAATATAAGGGTCAACGAAAAAATAAAGTGAAAAGTGTGATATGATG  
TATTTGGCTTTTGGCGCGCCTTGTGCGGCCCCGGCGATAACGCTGGGCGTGAGGCTGTGCCCCGC  
GGAGTTTTTTGCGCCTGCATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGA  
AGCAATAAGAATGCCGTTGGGGTTGCGATGATGACGACCACGACAACTGGTGTCAATTAT  
TTAAGTTGCCGAAAGAACCTGAGTGCAATTTGCAACATGAGTATACTAGAAGAATGAGCCA  
AGACTTGCGAGACGCGAGTTTGGCCGTGGTGCGAACATAGAGCGACCATGACCTTGAAG  
GTGAGACGCGCATAACCGCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCA  
GTATAAATAGACAGGTACATAACAACACTGGAATGGTTGTCTGTTTGAGTACGCTTTCAA  
TTCATTTGGGTTGCACTTTATTATGTTACAATATGGAAGGGAACCTTTACACTTCTCCTA  
TGCACATATATTAATTAAGTCCAATGCTAGTAGAGAAGGGGGTAACACCCCTCCGCGC  
TCTTTTCCGATTTTTTTCTAAACCGTGGAATATTTTCGGATATCCTTTTGTGTTTCCGGG  
TGTACAATATGGACTTCTCTTTTCTGGCAACCAAACCCATACATCGGGATTCTATAAT  
ACCTTCGTTGGTCTCCCTAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATA  
CCAGACAAGACATAATGGGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTG  
GTACATAACGAATACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTC  
ACTACCTTTTTCATTTGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTC  
TTTTTTTTTCTTTTCTCTCTCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAA  
ATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTG  
TTCCAGAGCTGATGAGGGGTATCTTCGAACACACGAAACTTTTTCTTCTCTTCACTCAG  
CACACTACTCTCTAATGAGCAACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAA  
TAAAAAAGTTTGGCGCTTGTCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTG  
TTTCTCTGTCATTGTTCTCGTTCCCTTTCTTCTTCTTCTTCTTCTTCTGCAATATTTCA  
AGCTATACCAAGCATACAATCAACTCCAAGCTTATGCCCAAGAAGAAGCGGAAGGTCTCG  
AGCGGCGCCAATTTAATCAAAGTGGGAATATTGCTGATAGCTCATTGTCTTCACTTTC  
ACTAACAGTAGCAACGGTCCGAACCTCATAACAACCTCAAACAAATCTCAAGCGCTTTCA  
CAACCAATTGCCTCCTCTAACGTTTCATGATAACTTCATGAATAATGAAATCACGGCTAGT  
AAAATTGATGATGGTAATAATCAAACCACTGTACCTGGTTGGACGGACCAAACTGCG  
TATAACGCGTTTGGAACTACTACAGGGATGTTAATAACCACTACAATGGATGATGTATAT  
AACTATCTATTGATGATGAAGATACCCCAACCAACCAAAAAAAGAGGGTGGGTGATC  
ACAAGTTTGTACAAAAAAGCAGGCTTGTGACCCCGGAATTGAGATCTACTAGTGCGGC  
CGCACGCGTACCCAGCTTTCTGTACAAAGTGGTGACGTGAGCTCCCTATAGTGAGTCG  
TATTACACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAAACCCGGTGAGCTCTAAGT  
AAGTAACGGCCGCCACCGCGGTGGAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTC  
TCCAATCAAGGTTGTGCGCTTGTCTACCTTGCCAGAAATTTACGAAAAGATGGAAGGG  
TCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAATAAGCGAATTTCTTATGATTTAT  
GATTTTTTATTATTAATAAGTTATAAAAAAATAAGTGTATACAAATTTTAAAGTGACTC  
TTAGGTTTTTAAAAAGAAATTTCTTGTCTTGTAGTAACCTTTCTCTGTAGGTGAGGTGCT  
TTCTCAGGTATAGCATGAGGTGCTCTTATTGACCACACCTCTACCGGCATGCCGAGCAA  
ATGCCTGCAAATCGCTCCCATTTACCCCAATTGTAGATATGCTAACTCCAGCAATGAGT  
TGATGAATCTCGGTGTGATTTTATGTCTCAGAGGACAATACCTGTTGTAATCGTTCTT  
CCACACGGATCCGCATCAGGCGAAATTTGTAACGTTAATATTTTGTAAAAATTGCGTTA  
AATATTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTAT  
AAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCA  
CTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGC-

CCACTACGTGAACCATCACCCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTA  
AATCGGAACCCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTG  
GCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCG  
GTCACGCTGCGCGTAACCACCACCCGCGCGCTTAATGCGCCGCTACAGGGCGCGTCC  
CATTGCGCCATTCACTGCA

FIGURE 98D



## pMAB86

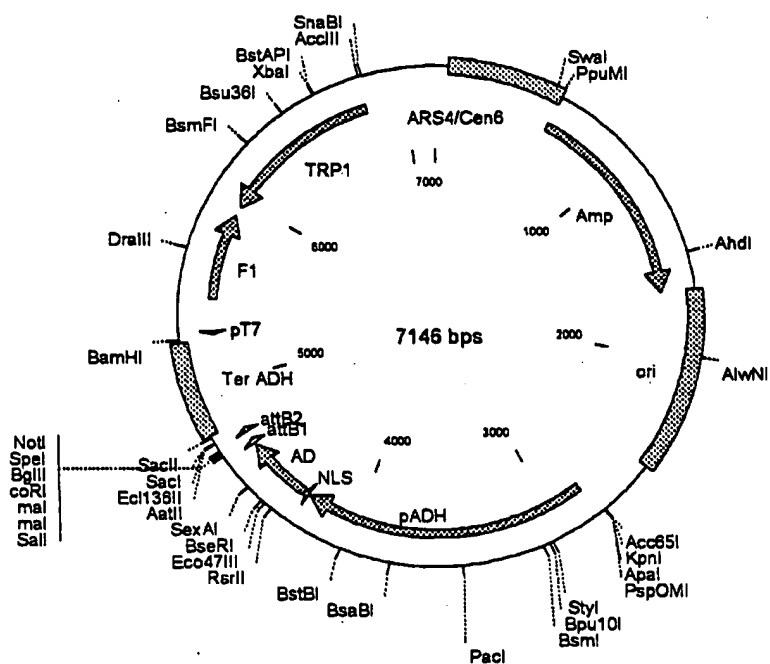


FIGURE 99A

pMAB86 7146 bp

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTT  
CTTAGGACGGATCGCTTGCCTGTAACCTACACGCGCCTCGTATCTTTAATGATGGAATA  
ATTTGGGAATTTACTCTGTGTTTATTTATTTTTATGTTTTGTATTTGGATTTTAGAAAGT  
AAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAAATAAACAAAGGTTTAAAAA  
ATTTCAACAAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATA  
GATATACATTGATTAACGATAAGTAAATGTAAATCACAGGATTTTCGTGTGTGGTCT  
TCTACACAGACAAGATGAAACAATTCGGCATTAAATACCTGAGAGCAGGAAGAGCAAGATA  
AAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAAAACAAAACT  
ATTTTTCTTTAATTTCTTTTTTACTTTCTATTTTTAATTTATATATTTATATTAATAA  
ATTTAAATTATAATTATTTTTATAGCACGTGAAAAGGACCCAGGTGGCACTTTTCGG  
GGAAATGTGCGCGGAACCCCTATTTGTTTTTCTAAATACATTCAAATATGTATCCG  
CTCATGAGACAATAACCCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGT  
ATTCAACATTTCCGTGTGCGCCTTATTCCTTTTTTTCGGCATTTTGCCTTCCTGTTTTT  
GCTCACCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG  
GGTTACATCGAAGTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAA  
CGTTTTCCAATGATGAGCACTTTTAAAGTCTGCTATGTGGCGCGGTATTATCCCGTATT  
GACCGCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATGACTTGGTGTGAG  
TACTCACCAGTACAGAAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGTCAGT  
GCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGA  
CCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGATCATGTAACCTCGCCTTGATCGT  
TGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTA  
GCAATGGCAACAACGTTGCGCAAACCTATTAAGTGGCGAACTACTTACTCTAGCTTCCCGG  
CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC  
CTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGT  
ATCATTGCGAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGAGC  
GGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTG  
ATTAAGCATTGGTAACCTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAA  
CTTCATTTTTTAATTTAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAA  
ATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGA  
TCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAACAAAAAAACCACCG  
CTACCAGCGGTGGTTTGTGTGCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAC  
GGCTTCAGCAGCGCAGATACCAATACTGTCTCTTCTAGTGTAGCCGTAGTTAGGCCAC  
CACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTG  
GCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCG  
GATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGA  
ACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCC  
GAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACG  
AGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGTCCTGTGCGGTTTCGCCACCTC  
TGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCC  
AGCAACGCGGCCTTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT  
CCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACC  
GCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGC  
CCAATACGCAAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCACGAC  
AGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACT  
CATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTG  
AGCGGATAACAATTTACACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATT  
AACCCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCCCCCTCGAGATCCGGGATCGA  
AGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATGAAGGCAAAAGACAAATATAAG  
GGTCGAACGAAAAATAAAGTGAAAAGTGTGATATGATGTATTTGGCTTTGCGGCGCCGA  
AAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCTGTGGCGGACCCGCGCTCTTGC  
CGGCCCCGGCGATAACGCTGGGCGTGAGGCTGTGCCCGGCGGAGTTTTTTGCGCCTGCATT  
TTCCAAGTTTACCCTGCGCTAAGGGGCGAGATTGGAGAAGCAATAAGAATGCCGGTTGG  
GGTTGCGATGATGACGACCACGACCACTGGTGTCTATTATTTAAGTTGCCGAAAGAACTTG  
AGTGCATTTGCAACATGAGTATACTAGAAGAAATGAGCCAAGACTTGCGAGACGCGAGTTT  
GCCGGTGGTGCGAACAAATAGAGCGACCATGACCTTGAAGGTGAGACGCGCATAACCGCTA-

FIGURE 99B

GAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCAGTATAAATAGACAGGTACATA  
CAACACTGGAAATGGTTGTCTGTTTGAGTACGCTTTCAATTCATTTGGGTGTGCACTTTA  
TTATGTTACAATATGGAAGGGAACCTTTACACTTCTCCTATGCACATATATTAATTAAAGT  
CCAATGCTAGTAGAGAAGGGGGGTAACACCCCTCCGCGCTCTTTTCCGATTTTTTCTAA  
ACCGTGGAATATTTCCGATATCCTTTTGTGTTTCCGGGTGTACAATATGGACTTCCTCT  
TTTCTGGCAACCAACCCATACATCGGGATTCTATAATACCTTCGTTGGTCTCCCTAAC  
ATGTAGGTGGCGAGGGGAGATATACAATAGAACAGATACCAGACAAGACATAATGGGCT  
AAACAAGACTACACCAATTACACTGCCTCATTGATGGTGGTACATAACGAACATAACTG  
TAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTCACTACCCTTTTTCCATTGACC  
ATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTCTTTTTTTTTCTTTCTCTCTC  
CCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAAATGATGGAAGACACTAAAGGA  
AAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTGTTCCAGAGCTGATGAGGGGTA  
TCTTCGAACACAGAAACTTTTTCTTCTTTCATTACGCACACTACTCTCTAATGAGCA  
ACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAATAAAAAAGTTTGCCGCTTTG  
CTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTGTTCCTCGTCATTGTTCTCGT  
TCCCTTTCTTCTTGTGTTCTTTTTCTGCACAATATTTCAAGCTATACCAAGCATACAATC  
AACTCCAAGCTTATGCCCCAAGAAGAAGCGGAAGGTCTCGAGCGGCGCCAATTTAATCAA  
AGTGGGAATATTGCTGATAGCTCATTGTCTTCACTTCACTAACAGTAGCAACGGTCCG  
AACCTCATAACAACCTCAAACAAATTCTCAAGCGCTTTCACAACCAATTGCCTCCTCTAAC  
GTTTCATGATAACTTCATGAATAATGAAATACGGCTAGTAAAATTGATGATGGTAATAAT  
TCAAAACCACCTTCACCTGGTTGGACGGACCAAACTGCGTATAACGCGTTTGAATCACT  
ACAGGGATGTTAATAACCACTACAATGGATGATGTATATACTATCTATTGATGATGAA  
GATACCCACCAAAACCAAAAAAGAGGGTGGGTGATCACAAGTTTGTACAAAAAGCA  
GGCTTGTGCGACCCCGGAATTGAGATCTACTAGTGCAGCGCCGACGCGTACCCAGCTTTCT  
TGTACAAAGTGGTGACGTCGAGCTCTAAGTAAGTAACGCGCCGCCACCGCGGTGGAGCTTT  
GGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTGCGCTTGTCTACCTT  
GCCAGAAATTTACGAAAAGATGGAAAAGGGTCAAATCGTTGGTAGATACGTTGTTGACAC  
TTCTAAATAAGCGAATTTCTTATGATTTATGATTTTATTATTAAATAAGTTATAAAAAA  
AATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTAAACGAAAATTTCTTGTTCTT  
GAGTAACTCTTCTGTAGGTGAGGTTGCTTCTCAGGTATAGCATGAGGTCGCTCTTAT  
TGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCCATTTACCCCA  
ATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGTATTTTATGTCTT  
CAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCCAATTGCGCCCTATAGTGA  
GTCGTATTACAATTCACCTGGCCGTCGTTTACAACGTCGTGACTGGGAAAACCCCTGGCGT  
TACCCAACTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAG  
GGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGACGCGCCC  
TGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTT  
GCCAGCGCCCTAGCGCCGCTCCTTTGCTTTCTTCCCTTCTTCTCGCCACGTTGCGCC  
GGCTTTCCCGTCAAGCTCTAAATCGGGGGTCCCTTTAGGGTTCCGATTTAGTGCTTTA  
CGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTACGTAAGTGGGCCATCGCCC  
TGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTG  
TTCCAAACTGGAACAACACTCAACCCCTATCTCGGTCTATTCTTTTGATTATTAAGGGATT  
TTGCCGATTTGCGCCTATTGGTTAAAAAATGAGCTGATTTAACAATAATTTAACGCGAAT  
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TTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTAATCTAAGCGCATCAC  
CAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGCTTTGCGGGCTCTCTT  
GCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTACCTGTCCACCTGCTT  
CTGAATCAAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTGCACTGAGTAGTATGT  
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CTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGTAATCATTGACCAGAG  
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AACTATTTTTATATGCTTTTACAAGACTTGAAATTTTCTTGAATAACCGGGTCAATTG  
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TGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTCTTTTTTCGACCGAAT-

TAATTCTTAATCGGCAAAAAAGAAAAGCTCCGGATCAAGATTGTACGTAAGGTGACAAG  
CTATTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTCATAACTGCAAAGTAC  
ACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATATATAGTAATGTCGTT  
TATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACC  
CGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGAC  
AAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGTCAGAGGTTTTACCGTCATCACCGAAAC  
GCGCGA

FIGURE 99D

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

REC'D

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>8</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30103
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pEZC15101)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float:right">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30100
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float:right">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pENTR-1A)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30102
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pENTR-3C)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30101
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pENTR-2B)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (If the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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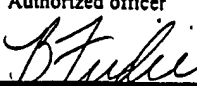
**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>WIPQ</u> <u>PCT</u> <u>20-21</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float:right">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30108
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float:right">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB10B(pCMVSPORT6)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30105
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pEZC15103)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (If the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30104
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pEZC15102)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>31</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float:right">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit <b>February 27, 1999</b>	Accession Number <b>NRRL B-30099</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float:right">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pAHPKan) or Escherichia coli DB3.1(pAttPKan)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (If the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p align="center">For receiving Office use only</p> <p><input checked="" type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer <b>Barbara Fridie</b> PCT Operations - IPD Team 1 703) 305-3777 703) 305-3230 (FAX)</p>	<p align="center">For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
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*Escherichia coli* DB3.1(pENTR-3C)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**NORWAY**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

**SINGAPORE**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-3C)**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-2B)

## AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

## DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

## FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pENTR-2B)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.



*Escherichia coli DB3.1(pENTR-2B)***SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-1A)

## AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

## DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

## FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pENTR-1A)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-1A)

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB10B(pCMVSPORT6)***AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

**FINLAND**

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*Escherichia coli* DB3.1(pAHPKan) or *Escherichia coli* DB3.1(pAttPKan)

## AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## CANADA

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## DENMARK

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## FINLAND

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*Escherichia coli DB3.1(pAHPKan) or Escherichia coli DB3.1(pAttPKan)*

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB3.1(pAHPKan) or Escherichia coli DB3.1(pAttPKan)*

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.



*Escherichia coli DB10B(pCMVSport6)***ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**NORWAY**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

**SINGAPORE**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB10B(pCMVSPORT6)*

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pEZC15103)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pEZC15103)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**NORWAY**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

**SINGAPORE**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pEZC15103)**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pEZC15102)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pEZC15102)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**NORWAY**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

**SINGAPORE**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB3.1(pEZC15102)***SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.



*Escherichia coli* DB3.1(pEZC15101)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

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*Escherichia coli* DB3.1(pEZC15101)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**NETHERLANDS**

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**NORWAY**

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**SINGAPORE**

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***Escherichia coli DB3.1(pEZC15101)*****SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

**UNITED KINGDOM**

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*Escherichia coli* DB3.1(pENTR-3C)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/05432

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : 435/91.2, 252.3, 320.1; 530/350; 536/ 23.1, 24.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/91.2, 252.3, 320.1; 530/350; 536/ 23.1, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P ---- Y,P	US 5,888,732 A (HARTLEY et al.) 30 March 1999, see entire document.	1-21, 25-30 36-38 ----- 22-24, 31-35
X - Y	HASAN et al. Escherichia coli genome targeting, I. Cre-lox-mediated in vitro generation of ori- plasmids and their in vivo chromosomal integration and retrieval. Gene. 1994, Vol. 150, pages 51-56, see entire document.	1-5, 10, 11, 19-21 ----- 15-18, 22-38
X - Y	KATZ et al. Site-specific recombination in Escherichia coli between the att sites of plasmid pSE211 from Saccharopolyspora erythraea. Mol. Gen. Genet. 1991, Vol. 227, pages 155-159, see entire document.	1-11, 19-21 ----- 15-18, 22-38

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

08 MAY 2000

Date of mailing of the international search report

23 MAY 2000

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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	ASTUMIAN et al. Site-specific recombination between cloned attP and attB sites from the Haemophilus influenzae bacteriophage HP1 propagated in recombination deficient Escherichia coli. J of Bacteriology. March 1989, Vol. 171, No. 3, pages 1747-1750, see entire document.	1-11, 19-21 ----- 15-18, 22-38

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International application No.

PCT/US00/05432

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

C07H 21/04; C07K 1/00, 14/00; C12N 1/21, 15/00, 15/09, 15/63, 15/70; C12P 19/34

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, STN (CAPLUS); DIALOG (MEDLINE, BIOSIS, SCISEARCH, PASCAL)

Terms: att (B?, P?, R?, L?), MCS, POLYLINKER, PLASMID, VECTOR, LOCALIZATION, SIGNAL, TRANSCRIPTION, TERMIN?, TRANSLATION?, ORI, REPLICON, GST, HEXHIST?, THIOREDOX?, CLEAVAGE, SITE?, SPECIF?, DIRECT?, RECOMBIN?, CLON?, INSERT?